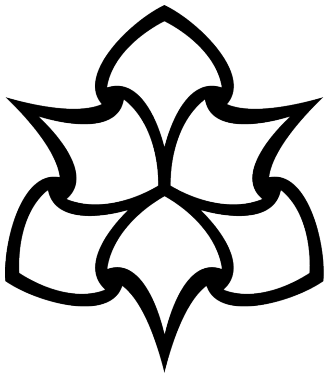

# Combined Effects of Ageing and Body Composition on Skeletal Muscle Structure and Function in Untrained Individuals

DAVID JONATHAN TOMLINSON

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Institute for Performance Research
Department of Exercise and Sports Science
Manchester Metropolitan University

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## Abstract

This thesis investigated whether body composition modulates neural, morphologic and/or functional characteristics of skeletal muscle in young (Y: 18-49 years) and old (O: 50-80 years), adult male (n=34) and female (n=102) populations. Classification by BMI revealed no significant differences in agonist muscle activation and antagonist co-contraction in Y and O individuals. However, high adiposity in Y (but not O) females led to lower muscle activation (94 vs. 88 %). Plantar flexion (PF) maximum voluntary contraction torque corrected for antagonist co-contraction and muscle activation (nMVC), gastrocnemius medialis (GM) muscle volume (MV) and physiological cross sectional area were significantly higher in the Y obese compared with that of underweight (+27%, +77% & +77%) and normal weight (+23%, +73% & +70%) females. No group differences were reported in the O female cohort. Fascicle pennation angle in Y and O females incremented with increasing BMI. PF nMVC normalised to GM MV (nMVC/GM MV) was lower in the Y obese females whether classified by BMI (-26%) or adiposity (-11%) in comparison to their normal weight counterparts. At the fascicular level, no impact of BMI or adiposity was evident in either Y or O female populations. There was an ageing-associated accelerated loss of muscle content in the obese (-2.1cm$^3$/year) and high adipose (-2.1cm$^3$/year) females. The continuum of both adiposity and BMI revealed similar impact on nMVC, GM MV and nMVC/GM MV, in both Y and O males. Interestingly, the slopes of the regressions implied the Y males to increase GM specific force (SF) with either increasing BMI or adiposity. Differential gender effects were observed at the fascicular level with the slope of GM SF being steeper in the obese males compared with the obese females (-0.373 N/cm$^2$/year vs. 0.056 N/cm$^2$/year; Student's *t*-statistic -4.56, $p<0.05$).

In conclusion increased adiposity (as evident in obesity) variably overloads the skeletal musculature in the Y compared to the O untrained adult population. However, obesity accelerates the magnitude of ageing-related sarcopenia and asthenia. The wider implication of this thesis relates to the need for the individualisation of strategies to modulate the functional and mobility limitations seen in older age.

## Publications

Tomlinson DJ, Erskine RM, Morse CI, Winwood K, Onambele-Pearson GL (2014) Combined effects of body composition and ageing on joint torque, muscle activation and co-contraction in sedentary women. Age 36 (3):9652. doi:10.1007/s11357-014-9652-1
(Data presented in Chapter 2)

Tomlinson DJ, Erskine RM, Winwood K, Morse CI, Onambele GL (2014) The impact of obesity on skeletal muscle architecture in untrained young vs. old women. J Anat 225 (6):675-684. doi:10.1111/joa.12248
(Data presented in Chapter 3)

Tomlinson DJ, Erskine RM, Winwood K, Morse CI, Onambele GL (2014b) Obesity decreases both whole muscle and fascicle strength in young females but only exacerbates the aging-related whole muscle level asthenia. Physiol Rep 2 (6). doi:2/6/e12030 [pii]10.14814/phy2.12030
(Data presented in Chapter 4)

## Conference Proceedings

Tomlinson DJ, Erskine RM, Morse CI, Winwood K, Onambele-Pearson GL. The Effect of Adiposity on Skeletal Muscle Size and Strength in Untrained Women. Poster session presented at the 37th Congress of the International Union of Physiological Sciences, Birmingham (2013).
(Data presented in Chapter 2)

Tomlinson DJ, Erskine RM, Morse CI, Winwood K, Onambele-Pearson GL. The Continuum of Adiposity and its Effect on Skeletal Muscle Size, Structure and Function in Untrained Young versus Old Males. Poster session presented at the Physiological Society Meeting (Obesity: A Physiological Perspective), Newcastle (2014).
(Data presented in Chapter 5)

Tomlinson DJ, Erskine RM, Morse CI, Winwood K, Onambele-Pearson GL. The impact of obesity on bone mineral density changes with ageing in sedentary women. *Prize winning* Poster presented at the British Society for Research on Ageing Annual Meeting. Liverpool John Moores University (2014).

## Contents

## Thesis outline

The structure of this thesis has been arranged into 7 chapters investigating any effect of body composition on muscle structure and function in both untrained men and women.

**Chapter 1** is a review of the current literature investigating the effect of obesity on muscle strength in adolescent, adult and elderly populations. The structure of the review starts with a discussion on the physiological and biomechanical determinants that effect maximum force generation and the rationale for controlling for these parameters when assessing muscle function. Thereafter, discussions are lead towards the current literature in the previously mentioned age classifications.

**Chapter 2** assesses the effect of body composition on agonist muscle activation using the interpolated twitch technique and antagonist co-activation using surface electromyography in untrained young and old females. This chapter segregates participants by both their body mass index (BMI) and body fat percentage when classifying obesity in order to examine between group differences. This approach is novel and furthers current knowledge in this topic through attempting to highlight the inherent limitations in the classification of obesity using a participant's BMI only.

**Chapter 3** assesses the impact of body composition on muscle architecture and size in untrained young and old females. Discussions within the chapter centre around the impact of muscle architecture on the pennate musculature of the lower limbs when quantifying muscle size and the potential erroneous conclusions when not accounting for this variable within the calculation of muscular functional capacity.

**Chapter 4** examines the impact of body composition on force normalised at both a gross (torque/muscle volume) and fascicular (GM specific force) levels in untrained young and old females categorised by BMI or by adiposity. This chapter also discusses the age related loss of the above-mentioned strength measures, when participants are categorised by BMI or by adiposity.

**Chapter 5** assesses the continuum of both BMI and adiposity against maximum voluntary contraction, gastrocnemius medialis muscle volume, intrinsic strength (torque/muscle volume), and gastrocnemius medialis specific force in untrained young and old males. This chapter additionally discusses the impact of BMI and

adiposity on the magnitude of ageing related sarcopenia and asthenia in a male population.

**Chapter 6** explores gender variations in the response to the continuum of adiposity and BMI when segregated by age classification. The influence of gender on the magnitude of ageing related sarcopenia and asthenia is discussed.

**Chapter 7** contains the general discussion and conclusion of the thesis, bringing together the impact of body composition on the neuromuscular and morphological determinants of maximal strength in obese male and female populations. Study Limitations and Directions for future research are also presented within the chapter.

# Chapter 1: Review of the Literature

## Introduction

The prevalence of obesity is a prominent public health concern. Within the UK the proportion of clinically obese adults has nearly doubled (13.6% - 26%) since 1996 (Zaninotto et al. 2009). From the latest health survey in England, just over a quarter of adults were classified as clinically obese (26% of all men and women) (NHS 2011) and, by 2025, these figures are predicted to rise to 46% of all men and 37% of all women (Foresight 2007). The associated problem with the rising level of obesity is the development of a variety of conditions, such as non-insulin dependent diabetes mellitus (Steppan et al. 2001), cardiovascular disease (Larsson et al. 1984), coronary heart disease (Manson et al. 1990), hypertension (Manicardi et al. 1986), stroke (Song et al. 2004) and cancer (Bianchini et al. 2002). However, additional to these co-morbidities, obesity has been shown to have a negative impact on skeletal muscle in adolescents (Blimkie et al. 1990; Maffiuletti et al. 2008), adults (Hulens et al. 2001; Maffiuletti et al. 2007) and the elderly (Zoico et al. 2004; Rolland et al. 2004)

Researchers have examined the effect obesity has on maximal isotonic (Lafortuna et al. 2005), isometric (Blimkie et al. 1990; Maffiuletti et al. 2007; Maffiuletti et al. 2008; Rolland et al. 2004; Hilton et al. 2008) and isokinetic (Blimkie et al. 1990; Maffiuletti et al. 2007; Hulens et al. 2002; Hulens et al. 2001; Delmonico et al. 2009; Hilton et al. 2008) strength in a variety of age classifications ranging from adolescents to the elderly. The majority of these studies agree that absolute strength is higher in obese compared to non-obese individuals, but the consensus between all studies is that strength is lower when normalised to total body mass. The implications for reduced strength relative to body mass are foremost relevant to an older population, as these are normally affected by a reduced functional capacity, (e.g. walking, climbing stairs, going down stairs, rising from a chair or bed and general movement difficulties) (LaRoche et al. 2011; Rolland et al. 2009) and an increased risk of joint pathologies (e.g. knee and hip osteoarthritis) (Cooper et al. 1998; Slemenda et al. 1998) and hence would have reduced quality of life. Therefore, understanding the adaptations of skeletal muscle caused through abnormally high levels of adiposity needs to be a priority, owing to the combination of a demography of increased prevalence of obesity supplemented with increased life expectancy (Kirkwood 2008).

It is possible that a greater decrease in relative strength in older obese people may partly be modulated via a higher state of systemic inflammation (Schrager et al. 2007), as fat deposits can act as endocrine organ secreting

various pro-inflammatory cytokines. This hypothesis has been demonstrated through obesity-related increases in pro-inflammatory cytokines, specifically interlukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) (Schrager et al. 2007). Cytokines are in fact associated with lower muscle mass and strength in the elderly (presumably through stimulating muscle protein catabolism and inhibiting muscle protein synthesis) (Visser et al. 2002). Yet, the specific effect that chronically high levels of adiposity combined with ageing-associated systemic inflammation may have upon skeletal muscle structure and function is yet to be fully understood. Therefore, the aim of this chapter is to review the current literature on the link between adiposity and skeletal muscle force and power generation in adolescents, young adults and the elderly, assessing the evidence of any adaptations on skeletal muscle and critically analysing the methodologies employed.

## 1. Physiological and biomechanical components regulate the muscle's ability to generate maximum force

When examining the effect obesity has upon skeletal muscle structure and function, there is the need to understand the **physiological** and **biomechanical** components that regulate maximum strength generation irrespective of gender, age or body composition. The physiological component can be split into two distinct categories: **neurological** and **morphological.**

The **neurological** component comprises of various mechanisms that control muscle contraction including motor unit recruitment, firing frequency, synchronisation of motor units and antagonist co-activation. The importance of measuring these neuromuscular variables when considering the force capabilities of skeletal muscle is the acceptance that both untrained young and old individuals do not fully activate their muscles during maximal contractions (Erskine et al. 2010b; Morse et al. 2004), and not taking this into account could potentially lead to underestimating skeletal muscle force generating capabilities. These variables have been shown to alter following progressive resistance training in both the young and old, through a combination of increased motor unit recruitment and/or firing frequency, optimised synchronisation of the synergist muscles and decreased antagonist co-activation (Hakkinen et al. 1998; Milner-Brown and Stein 1975; Moritani and deVries 1979; Reeves et al. 2004b).

Whilst the need to quantify agonist muscle activation is highlighted in both the young and old untrained individuals, antagonist co-activation additionally has been shown to affect net agonist muscle force (Maganaris et al. 2001). If no correction were made for antagonist co-activation, the resultant force would be overestimated, as antagonist co-activation lowers the resultant agonist force through creating an opposing negative moment to the agonist contraction. Antagonist co-activation has been reported to be higher in older adults when compared to their younger counterparts (Macaluso et al. 2002; Hakkinen et al. 1998), yet following resistance training this been shown to decrease to levels comparable to their younger counterparts. This though remains a contentious subject as some studies report no significant differences between young and old individuals (13.5% vs. 9.6%) (Morse et al. 2005b). A rationale for increased antagonist co-activation in the elderly may be due to the need to stabilize the joint (Baratta et al. 1988), as seen in stair negotiation (Hortobagyi and DeVita 2000). This would be interesting to investigate in the obese individuals who potentially may need to stabilize the joint as a protective mechanism to minimize stress placed upon the joint due to the extra mass brought about by high body mass.

The **morphological** component that regulates the maximum amount of force generated by skeletal muscles is governed by numerous characteristics of which are muscle architecture (skeletal muscle fascicle length and pennation angle), muscle size, fibre type/myosin heavy chain composition, packing density of myofilaments and tendon properties.

The first component to discuss is muscle architecture and its importance when examining the functional significance of the pennate muscles in the lower limb (e.g. quadriceps femoris and triceps surae complex). Both the quadriceps femoris muscle group and triceps surae complex have muscle fascicles that are inserted into the aponeurosis at an angle, known as pennation angle. An increase in this angle, allows for more contractile material (sarcomeres) to be attached to the aponeuroses within the skeletal muscle, thus increasing the muscles maximal strength capability through (i) increased number of fibres that can be ‘packed’ into a volume of muscle (Aagaard et al. 2001) and (ii) the laws of trigonometry, since there is a net positive effect from an increased pennation angle up to 45 degrees (Alexander and Vernon 1975). Muscle fascicle pennation angle has been reported to increase following progressive resistance training in both young (Erskine et al. 2010b) and old individuals (Morse et al. 2007), thus partially explaining the increase in resultant torque. In addition, following simulated microgravity a

decrease in fascicle pennation angle accompanies the resultant lower maximal strength (de Boer et al. 2007), demonstrating that plasticity in architecture is linked to the stimulus placed upon the muscle structure. Similarly, ageing is characterised by a decrease in muscle fascicle pennation angle (Morse et al. 2005b). The need to examine muscle fibre pennation in the presence of obesity in both young and elderly individuals, is motivated by the timeliness of explaining any cumulative impact of increased adiposity and ageing especially when examining in the lower limb (e.g. Quadricep femoris and Triceps surae), in an ageing and more obese-prone society.

In conjunction with the pennation angle is the length of fascicle itself, which directly affects the shortening velocity of the muscle (sarcomeres in series). An increase in fascicle length has been reported following resistance training in both young (Seynnes et al. 2007) and old (Reeves et al. 2004b) individuals and is shown to increase maximal power output (Aagaard et al. 1994). As with fascicle pennation angle, a decline in fascicle length has been reported during the ageing process. A reduction in both the length and the pennation of fascicles explains the decrease in power output (itself linked to a lowering of both the shortening velocity and the torque), seen in the elderly (Thom et al. 2005). The ageing-related decrease in power, thus makes daily tasks that require dynamic power more difficult, including locomotion, lifting heavy objects, standing from a chair and stair negotiation.

The next morphological component that plays a role in regulating the strength capabilities of skeletal muscle is muscle size. The measurement of muscle size is quantified using a number of different methodologies including anatomical cross sectional area (ACSA), muscle volume or physiological cross sectional area (PCSA). However, of the three methodologies, the gold standard in the measurement of muscle size is PCSA (muscle volume/fascicle length), as this takes into account both muscle architecture and volume. Yet, even though PCSA is seen as the gold standard in the measurement of muscle size, ACSA has widely been used to quantify training adaptations brought about through resistance training, ageing and/or gender differences in both young (Narici et al. 1989) and old (Ferri et al. 2003) individuals. ACSA is defined as the area of muscle perpendicular to the longitudinal axis of the limb (Erskine et al. 2010b), yet the limitation of this measure when examining a pennate muscle (e.g. quadriceps and triceps surae), is that it does not account for muscle fascicle length and pennation angle. The implication for this is a potential underestimation of the PCSA of

pennate muscles (Alexander and Vernon 1975). Differences in the criterion measure therefore accounts, at least partly, for variations in degree of reported difference in muscle size between obese and non-obese individuals. Interestingly the majority of obesity related studies have used the pennate musculature of the knee extensors (Abdelmoula et al. 2012; Blimkie et al. 1990; Hulens et al. 2001; Maffiuletti et al. 2008; Maffiuletti et al. 2007; Rolland et al. 2004) to investigate how obesity impacts on muscle structure. These studies have in fact utilised ACSA (Blimkie et al. 1990), total fat free mass (Maffiuletti et al. 2008) and leg lean mass (Abdelmoula et al. 2012) as a measure of muscle size to relate to knee extensor torque, and have thus not taken the architectural component of muscle into account.

The final structural component to be discussed is the fibre type composition of skeletal muscle and its subsequent impact on force generating capabilities. Fibre type composition is characterised by slow twitch (type I) or fast twitch (type IIa & b) muscle fibres. Slow twitch fibres are defined by their fatigue resistant properties coupled with lower maximal power output compared to the fast twitch isoforms (Bottinelli et al. 1996). Fast twitch fibres are characterised by lower fatigue resistance and a greater shorting velocity and maximal power output compared to type I fibres (Bottinelli et al. 1996). During the ageing process, there is preferential atrophy of type II fibres thus impacting on an individual's maximum torque capability, lowering the intrinsic force of the skeletal muscle as a whole (Lexell et al. 1988). However, obese individuals have been shown to possess a higher percentage of type II fibres in comparison to lean counterparts. Indeed following gastric band surgery, the proportion of type I fibres was shown to increase in proportion to the extent of weight loss (Tanner et al. 2002). It may be rationalised from a hypothesis whereby the loading of increased adiposity would be similar to that elicited through traditional resistance training (where fast to slower fibre type transition from IIb-to-IIa) (Staron et al. 1994), that, a lower force output capacity resulting from greater proportion of slower twitch fibres (Bottinelli et al. 1996), could partially explain the lesser relative strength to body mass observed in obese individuals (Blimkie et al. 1990; Maffiuletti et al. 2007; Maffiuletti et al. 2008; Rolland et al. 2004; Hilton et al. 2008).

The **biomechanical** component that regulates the muscles maximal force generating capacity can be characterised by the limb's tendon moment arm length, tendon mechanical stiffness and skeletal muscle length during contraction. The tendon moment arm length is defined as the perpendicular distance from the

centre of rotation to the line of action at the tendon, which acts as the leverage for effective force transmission from the contracting muscles about a joint (Tsaopoulos et al. 2006). The implications of the tendon moment arm in relation to the force generating capability of an individual can be explained by a larger moment arm of an individual allows for them to create a greater joint moment, thus demonstrating the need to accurately quantify the moment arm length. The potential variability of tendon moment length between individuals is due to possible inter individual differences in the physical variation in bone anthropometrics (Maganaris et al. 2001; Stebbings et al. 2013). However, in addition to the need of accurately quantifying tendon moment arm length, is the necessity to determine the material properties of the tendon. The tendon itself is responsible for the transmission of the contractile effort of the working muscle to the joint. Thus, its mechanical stiffness has implications in the expression of maximal muscle force and power. Ageing has been shown to lower mechanical stiffness (i.e. making tendons more compliant), thus influencing the rate of torque development (Onambele et al. 2006a). The detrimental aspect of this characteristic for an ageing individual is the inability to quickly reach maximal torque, potentially increasing the likelihood of falls (Karamanidis et al. 2008) and thus potential fractures. Knowledge of these two components of tendon characteristics helps in quantifying skeletal muscle force from knowledge of external torque. This would then allow investigators to calculate the intrinsic force capabilities of the skeletal muscle (specific force) (Maganaris et al. 2001), depicting the true effect of a stimulus placed upon an individual.

The next biomechanical component to impact on maximal strength is the length of the muscle during contraction. This relationship is known as the length-tension relationship and is directly related to the number of cross-bridges (i.e. actin-myosin filaments interaction) in a sarcomere during a contractile effort. The length tension relationship has been shown to follow a parabolic relationship where optimum strength generation occurs over a relatively small range of muscle lengths (the plateau or optimal muscle length), either side of which the strength potential decreases (Hawkins and Bey 1997). Interestingly the length-tension relationship can be altered through preferentially training at a specific muscle length (Jones et al. 1989). This however may have negative implications for individuals who adapt daily activities to work with small ranges of joint angles (in an attempt to minimise discomfort from potential osteoarthritis or mobility

limitations), as they would then struggle to work at the extremes of range of motion (e.g. deep knee bend when getting up from the floor or low seat).

In summary, in order to gain a true reflection of the impact that high body mass as seen in obesity (and hence high levels of adiposity) places on skeletal muscle, there is a distinct need to correct for the physiological and biomechanical determinants that regulate skeletal muscle strength capacity. By not accounting for the neuromuscular and biomechanical determinants of strength, this potentially under/overestimates the contractile characteristics of the skeletal muscles of an individual.

Not withstanding the limitations of the existing literature, the following is a description of the current findings in relation to the impact of obesity (and/or body composition), on skeletal muscle structural and contractile characteristics.

## 2. Does the additional loading of adiposity seen in obesity act as a training stimulus on skeletal muscle?

Investigations into the effects of obesity on muscle size and function have described the inter-link between muscle torque and power to body mass, where obese people elicited higher absolute maximum voluntary contraction (MVC) torque and power than normal-weight individuals (Blimkie et al. 1990; Lafortuna et al. 2005; Hulens et al. 2001; Maffiuletti et al. 2007; Abdelmoula et al. 2012; Maffiuletti et al. 2008). A rationale for higher absolute MVC torque and power in obese individuals is from the suggestion by Thoren *et al.* (1973) that extra mass from high levels of fat mass seen in obese individuals might elicit a positive training stimulus on skeletal muscle. This hypothesis was strengthened by Bosco *et al.* (1986) who reported increases in muscle power in anti-gravity muscles following 3 weeks of simulated hypergravity through the use of weighted vests. The weighted vests (7-8% body mass) utilised in this study were worn from morning until evening similar to the excess (fat) mass an obese individual would carry around daily. The causative explanations given for the increase in performance were through changes in neurogenic mechanisms by increased motor unit firing rate, additional recruitment of motor units and the synchronisation of these motor units. These specific neural adaptations are accepted to occur in the initial phase of resistance training, with hypertrophy becoming the dominant factor after 3-5 weeks (Moritani and deVries 1979). Therefore, this poses the question that if individuals are obese for numerous years, would this create a

protective/favourable adaptation to the lower limbs through carrying higher inert mass (i.e. adipose tissue) during daily activities?

## 3. The effect of obesity on muscle strength and structure in adolescent individuals

By assessing both neural and muscular components of force generating capacity, Blimkie *et al.* (1990) were the first to extensively examine skeletal muscle performance in obese and non-obese adolescent males. Obesity was classified in the adolescent males using body fat percentage instead of body mass index (BMI; mass (kg)/height$^2$ (m)). The classification of obesity between the two groups was through using cut off points of estimated body fat% of below 20% as non-obese and above 30% as obese. Assessing body fat % was conducted using the sum of four skinfolds sites (subscapular, supra-iliac, triceps and biceps) and applying the equation by Durnin and Rahaman (Durnin and Rahaman 1967) to estimate body fat % in adolescent males. Yet, Blimkie *et al*. (1990) stated a problem with using this method, was that the age range of the population that gave rise to the original equation was lower than the population utilised in their own study (14.7yrs vs. 16.6yrs). Lohman (1986) stated this to potentially overestimate body fat % and underestimate lean body mass. Additionally, this method has been shown to have limited accuracy in quantifying % fat mass in individual adolescents compared with the "gold standard", dual energy x-ray absorptiometry (DEXA) (Rodriguez et al. 2005). This is due to the Durnin and Rahaman method (1967) systematically exhibiting higher %fat values than would be obtained from a DEXA scan (+10.6). In addition the correlation between % bias and fatness was significant ($r=0.53$; $p<0.001$), which translates into heteroscedasticity in the residuals. In other words, the Durnin and Rahaman equation overestimates fat content in low body fat % adolescent males and underestimates fat content in those with a high body fat % (Rodriguez et al. 2005). Nevertheless, Blimkie *et al*. (1990) had chosen a more reliable method to assess obesity in children/adolescents than merely using a child's BMI (Taylor et al. 2002). Indeed BMI does not distinguish the difference between fat, lean tissue and bone. A DEXA directly measures the absorbance rate of x-rays and relates these to fat, bone and fat-free mass tissues, thereby giving an accurate measure of an individual's body composition.

The main observation from the Blimkie study was the reduced quadriceps femoris muscle activation in obese compared to non-obese adolescent males (85.1 vs. 95.2%; 100% = complete voluntary muscle activation). The obese adolescents studied by Blimkie *et al*. (1990) were outpatients at a children's exercise and nutrition centre, while the non-obese adolescents were selected from a local secondary school. It is not known whether the two cohorts were matched for habitual physical activity levels. Any potential differences in physical activity may have explained some of the variability in neuromuscular variables, such as agonist muscle activation and antagonist muscle co-activation (Martinez-Gomez et al. 2011; Moliner-Urdiales et al. 2010; Ramsay et al. 1990), which may have also confounded any potential difference in strength between obese and non-obese boys (Blimkie et al. 1990). Notwithstanding potential differences in the habitual physical activity background of the study participants, the data suggested that relative to their non-obese counterparts, obese adolescents had poorer neural activation capacity and/or sub-optimal motoneurons firing frequency, leading to a reduction in the degree of muscle fibre recruitment. Yet it has been shown in an adult population that high levels of visceral adiposity is associated with increased neural sympathetic drive (Alvarez et al. 2002). This increase in sympathetic nerve activity is additionally linked with metabolic syndrome and insulin resistance in obesity (Grundy 2004). This may provide a link to obesity and reduced motor performance through mechanisms such as peripheral neuropathy characterised by nerve damage evident in individuals with metabolic syndrome (Isomaa et al. 2001).

Interestingly in the Blimkie *et al*. (1990) study, there were no between group (obese vs. non-obese) differences in absolute isometric strength at a variety of muscle lengths (20°, 40°, 60°, 90° of knee extension) or in isokinetic knee strength (30°/s, 60°/s, 120°/s and 180°/s), when strength was made relative to quadriceps cross sectional area. These results differ from later work by Maffiuletti *et al.* (2008), who reported significantly higher absolute voluntary isometric strength in the obese adolescent at short muscle lengths (+25% at 40° extension) and during isokinetic efforts (+16%).

The strength in the design of this study was the control of physical activity in the adolescent males, as the exclusion criteria stated that no individual took part in rigorous physical activity and undertook less than 2 hours per week of recreational physical activity. The methodological differences between this study (Maffiuletti et al. 2008) and Blimkie et al. (1990) laid in accounting for the physical activity level

of participants, which would have in turn accounted for differences in reported impact of obesity between the two studies. As, it has been shown that vigorous levels of physical activity can increase strength in the anti-gravity muscles of the lower limb (Moliner-Urdiales et al. 2010). However, Maffiuletti *et al*. (2008) proposed that a rationale for significantly higher strength at short muscle lengths in the obese adolescent cohort could be their preferentially working at shorter muscle lengths to avoid excessive stress during an activity/sport or to avoid injury. Such a habitual loading protocol would shift the muscle length relationship to the left, hence placing obese adolescents at a disadvantage in daily activities involving a wider range of movement (e.g. deep squatting, getting up from a chair, walking fast, bending). This disadvantage would be further exacerbated by a lower relative muscle strength to body mass.

Maffiuletti *et al.* (2008) also looked at the effect of obesity upon voluntary compared with evoked muscle fatigue of the quadriceps during 50 repeated voluntary isokinetic leg extension contractions (180°/s) and 60 evoked isometric contractions (60° of knee extension). There were no differences reported between cohorts in both the voluntary and evoked fatigue protocols. Thus, Maffiuletti *et al.* (2008) suggested there was no reason to assume central and peripheral mechanisms of muscle fatigue differed between obese and non-obese adolescents. However, due to lower relative strength to body mass, obese individuals would have to work at a higher percentage of their maximum strength during activities that involved repetitive knee bending which may potentially increase mental and physical fatigue. This may potentially be a limitation of this specific test, as it was not undertaken in conditions that mimic the stress placed on muscle during a loaded movement such as body weight squats.

Similarly to Maffiuletti *et al.* (2008), Abdelmoula *et al*. (2012) reported higher absolute maximum isometric knee extension torque (+24% at 60° of extension) and lower MVC torque relative to body mass (-25%) in obese compared to non-obese adolescent males. Interestingly, Abdelmoula *et al*. (2012) reported higher MVC isometric torque normalised to thigh lean mass (+17.9%) and estimated thigh muscle mass (+22.2%). This differs from reports by both Blimkie *et al.* (1990) and Maffiuletti *et al.* (2008), who reported no significant differences in MVC torque normalised to quadriceps ACSA (Blimkie et al. 1990) or fat free mass (Maffiuletti et al. 2008). The discrepancies within these studies may be due to differences in the methodology in assessing thigh/quadriceps muscle mass. As mentioned above, the gold standard in the assessment of muscle size is the PCSA (as it accounts for

the pennate architecture of the quadricep femoris muscle group). However, Abdelmoula *et al*. (2012) attributed the higher strength relative to estimated muscle mass in their study sample, to higher agonist muscle activation and lower antagonist muscle co-activation in the obese adolescents. Their assumptions were in fact erroneous since the neural factors alluded to, had not actually been measured, and in fact, the obese adolescents had lower habitual physical activity levels, which one would normally expect to lead to a lowering in muscle activation capacity (Martinez-Gomez et al. 2011). Indeed as reported earlier, Blimkie *et al*. (1990) found muscle activation to be significantly lower in obese adolescent boys. Abdelmoula *et al*. (2012) also proposed that there could have been an increase in the contribution from the synergistic muscles in obese adolescent boys. Whether there may be an obesity-induced alteration in muscle recruitment strategy in young adolescents has yet to be demonstrated. We would propose instead, that an alternative rationale for the higher strength values relative to estimated muscle mass in the Abdelmoula study (2012) maybe differences in the intrinsic properties of the skeletal muscle of the two cohorts. In support of this hypothesis, previous research demonstrates an increase in fast twitch fibres in obese 26-62 year old adults (Kriketos et al. 1997). Such an effect, however, has yet to be confirmed in an obese adolescent population.

In summary, the general consensus is that obese adolescents elicit lower relative strength to body mass. Yet discrepancies exist when examining the absolute strength of obese vs. non-obese adolescents and strength relative to muscle mass. These differences between studies maybe attributed to variability in the methodology, including the control of habitual physical activity difference between participant groups, and/or the methods utilised in the quantification of muscle size. Interestingly, it has previously been reported that obese adolescents have lower agonist voluntary muscle activation. The implication of this is the potential to underestimate the strength capabilities of obese adolescents in studies not correcting for this variable. Yet, no study to date has examined the effect of antagonist co-activation has upon maximal torque output in obese vs. non-obese adolescent individuals thus potentially leading to further underestimating the quality of the muscle exposed to obesity. Further research in adolescents should focus on examining the variables that affect strength production such as agonist muscle activation, antagonist co-activation, physiological cross sectional area and moment arm length (O'Brien et al. 2010).

## 4. The effect of obesity on muscle strength and structure in young and old adults

One of the first studies to investigate the effects of obesity on muscle strength in an adult population was conducted by Hulens *et al.* (2001) in which the authors examined the effect obesity had on females' upper body (i.e. unloaded muscles (handgrip strength)), and anti-gravity (i.e. isokinetic knee and trunk extension, flexion and rotation strength) muscles and seemed to suggest an obesity 'advantage' in terms of absolute muscle strength for loaded musculature. This was demonstrated by the obese females having significantly higher isokinetic knee extension, trunk extension, flexion and rotational torque than the lean individuals, whilst no interaction existed in handgrip strength between cohorts. The study participants consisted of 80 lean individuals (BMI=22 ± 2) and 173 obese persons (BMI=38 ± 5). Whilst the mean age of the cohort was 39 years, the large age range (20-65 years) may have confounded any effect of obesity on skeletal muscle phenotypes. Indeed ageing is associated with changes in neuromuscular variables, such as a reduction in agonist muscle activation (Morse et al. 2004), a reduced muscle PCSA and volume (Morse et al. 2005b), and a decrease in muscle strength (Morse et al. 2005b). This is demonstrated in a separate study, in which Hulens *et al.* (2002) accounted for the confounding age factor by dividing their participants into two cohorts; 18-40 yrs vs. 41-65 yrs, and reported that the older obese cohort had significantly lower knee extension isokinetic MVC torque than their aged-matched leaner counterparts.

Hulens *et al.* (2001) had in fact reported that the loaded antigravity muscles of the knee extensors, back extensors and oblique abdominals were stronger in the obese compared to the lean women. Yet, when normalised to fat free mass (FFM), maximum knee extensor strength was significantly 6-7% lower in the obese cohort. The discrepancy regarding absolute MVC torque and MVC torque normalised to FFM may be due to lower agonist muscle activation [as seen in adolescents (Blimkie et al. 1990)]. Lower activation of motor units during a maximal contraction would potentially lower maximal strength generation resulting in both lower absolute and normalised MVC. Other explanations for the discrepancy of MVC torque relative to FFM could be the use of FFM instead of muscle volume or PCSA to accurately assess MVC torque relative to muscle size, due to it demonstrating an accurate in vivo representation of the maximum number of parallel-aligned sarcomeres. Interestingly, Hulens *et al.* (2001) reported no

differences in handgrip strength between obese and non-obese individuals opposite to his findings on absolute knee extensor strength, suggesting the additional body mass may act as a training stimulus, i.e. overloading the anti-gravity muscles in a similar way that performance has been shown to increase with the use of a weighted vest (Bosco et al. 1984). Hulens *et al*. (2001) supported this finding by demonstrating MVC torque relative to FFM during knee flexion was 18-20% lower in obese vs. non-obese individuals, while MVC knee extension torque was only 6-7% lower, suggesting that the additional body mass acted to favourably load the quadriceps over the hamstrings muscles.

Lafortuna *et al.* (2005) went further to examine gender differences in body composition, muscle strength and power output in 95 morbidly obese adults (28 men and 67 women) aged 29 ± 7 years. Body composition was analysed with bioelectrical impedance, while muscle strength of both the upper and lower limbs was assessed using isotonic gym equipment (chest press and leg press) and power output assessed by a standing vertical jump.

The main findings of the study by Lafortuna *et al.* (2005) revealed that obese young men were significantly stronger in both upper and lower limbs and more powerful than the obese young women, and these differences were attributed to greater FFM in the men (77.7 kg vs. 52 kg), a result which was expected since males tend to demonstrate this effect even in non-obese young adult populations (Janssen et al. 2000). Interestingly, when isotonic strength was normalised to FFM, all differences disappeared between genders. In terms of lower limb power output normalised to FFM, data showed obese males to have lower relative power to FFM than their normal weight counterparts and in addition it showed a strong though non-significant trend for a gender effect ($p=0.059$). This could be caused by a change in the intrinsic properties of the skeletal muscle of the lower limb, through a shift in fibre type composition to slower twitch fibres. This further supports the theorem that high body mass loads the antigravity muscles similarly to resistance training, thus causing a fast to slow transition in fibre type composition (Staron et al. 1994). This is demonstrated by a mean difference of 20 kg more inert body mass seen in the obese males than the obese females (128kg vs. 108kg) acting as potential enhanced loading stimulus during daily living activities. The isotonic upper body strength measures reported in this study support this hypothesis, as no differences were reported in upper body strength between normal weight and obese participants irrespective of gender, yet

significant strength differences were observed in the anti-gravity muscles during the leg press efforts.

Over and above differences in maximum quadriceps muscle strength, Maffiuletti *et al*. (2007) investigated differences in the fatigability of the quadriceps muscle between lean and obese male individuals. They examined the effect of obesity on isokinetic torque at 3 angular velocities (60, 120 and 180°/s), isometric maximum voluntary contractions (MVC) at 3 joint angles (40, 60 and 80°), a voluntary fatigue protocol of 50 consecutive isokinetic contractions (180°/s) and a stimulated fatigue protocol consisting of a modified Burke protocol (Burke et al. 1973) of 60 stimulations (60° of knee flexion) over a period of 5 minutes. The estimation of FFM and body fat % was conducted using bioelectrical impedance. However within severely obese individuals, the use of bioelectrical impedance can potentially underestimate body fat thus overestimate FFM through the use prediction formulas used for normal weight individuals that assume a constant hydration status, uniform body water distribution and body morphology similarities (Deurenberg 1996).

Maffiuletti *et al*. (2007) reported the obese males to have higher absolute torque at all angles and velocities suggesting that in an adult population, obese individuals did not favourably work at a specific muscle length, contrary to adolescents who had higher absolute strength at short muscle lengths (Maffiuletti et al. 2008). At their optimal angle and peak velocity obese individuals had 16% and 20% higher absolute isometric and isokinetic torque respectively, compared with their non-obese counterparts. Yet, when normalising absolute strength to body mass, they found that maximum isometric and isokinetic knee joint torque were respectively 34.5% and 32.5% lower than in normal-weight individuals similar to previous work in an adult population (Hulens et al. 2001; Lafortuna et al. 2005). However, when both isometric and isokinetic MVC torque were normalised to FFM, any significant differences between cohorts disappeared. It should be noted that the standardisation of MVC torque to total FFM does not differentiate the quadriceps femoris muscle group from other muscle groups, hence extraneous synergistic/antagonistic muscles to knee extension efforts would have confounded the authors' concluding remarks. Furthermore, MVC joint torque is influenced by additional factors such as voluntary muscle activation capacity, antagonist muscle co-activation and the tendon moment arm (Erskine et al. 2009), which were not considered in this study. To date though, no study has accounted for these

differences within the current literature in an adult population when comparing obese to non-obese.

Remarkably, Maffiuletti and colleagues (2007) reported that following 50 isokinetic voluntary contractions the loss in torque was significantly greater in obese compared to normal weight adults (-64% vs. -51%). There was no difference in torque reduction following the evoked protocol (-25% mean torque loss). This suggests that obese adults demonstrate greater central/motivational limitations regarding fatigue, while the peripheral fatigability of the muscle is no different to that of non-obese adults.

Further research by Maffiuletti *et al*. (2005) reported that obese individuals have inadequate postural stability when compared to lean persons. These balance issues were improved after a few postural stability-training sessions during a body weight reduction programme. This finding has implications for the prevention of falls, especially in obese elderly individuals who are more at risk of falls and fractures (Himes and Reynolds 2012). Interestingly, the majority of studies investigating the effect of obesity on muscle strength have focussed on the knee extensors. Yet, as demonstrated by Maffiuletti *et al*. (2005), the contribution of the plantar flexors during postural stability (Onambele et al. 2006a) suggests more work should focus on this muscle group when examining the effect of obesity on muscle function.

The only study to focus on the plantar flexors is that of Hilton *et al*. (2008). Contrary to the findings of Hulens *et al.* (Hulens et al. 2002; Hulens et al. 2001), Lafortuna *et al*. (2005) and Maffiuletti *et al*. (2007), Hilton *et al*. (2008) reported that MVC torque and lower limb power were lower in obese compared to non-obese people, both in absolute terms and when power was normalised to muscle volume. Importantly to note, is that the sample size of this study was small (n = 6; BMI = 36 ± 8 vs. n = 6; BMI 28 ± 6) and the obese subjects in this study had diabetes mellitus (DM). This condition is strongly associated with obesity (Mokdad et al. 2003), as well as peripheral neuropathy, which is characterised by nerve damage, leading to reduced neural function. Studies have shown that DM sufferers [many of whom are overweight (Steppan et al. 2001)] can develop peripheral neuropathy (Young et al. 1993), which is thought to be associated with chronic hyperglycaemia and hyperlipidaemia (Tesfaye et al. 2005) and is characterised by motor dysfunction (Andersen et al. 1997) and reduced strength (Andersen et al. 1996). In other words, as increased adiposity is associated with insulin resistance and impaired glucose tolerance (Steppan et al. 2001), it is

possible that body fat composition maybe related to the level of voluntary muscle activation. This is supported by the finding that muscle activation was lower in obese vs. lean adolescents (Blimkie et al. 1990). Nevertheless more research is necessary to confirm if this is also the case in adults, and to what degree ageing may impact on any association between obesity, neuropathy and muscle-strength.

In summary, an analysis of studies in an adult population suggests that obese individuals have significantly higher absolute strength, but lower strength normalised to body mass in the antigravity muscles of the lower limb. However, upper limb strength data reveals no statistical difference between obese and normal weight individuals. This suggests the loading brought about through higher inert mass (increased adiposity) simulates a resistance-training stimulus, but only specifically to the weight bearing (i.e. antigravity) musculature. Interestingly, when absolute strength measures are made relative to FFM, all significant differences between obese and non-obese cohorts are erased in the majority of cases. However, the use of total body FFM (instead of using PSCA) does not account for the pennate architecture of the knee extensors or plantar flexors, thus potentially confounding the statistical differences between cohorts. Interestingly, the combined effect of obesity coupled with the co-morbidities of DM highlights the detrimental effect of adipose tissue in large quantities in terms of lowering absolute and relative strength through motor dysfunction, and thus negatively impacting on activities of daily living.

## 5. The interaction between age and obesity, and its effect on skeletal muscle (sarcopenic obesity)

The age related loss of skeletal muscle mass and function has been termed “sarcopenia”, (Narici and Maffulli 2010; Rosenberg 1997). Sarcopenia has been shown to increase the risk of developing functional limitations (e.g. Walking and climbing stairs) and physical disabilities as defined by the difficulty in performing daily activities (e.g. shopping, household chores and making meals) (Janssen et al. 2002). Therefore, minimising the incidence of sarcopenia and maintaining functional mobility is paramount to ensuring a good quality of life. There does not appear to be a single cause for sarcopenia as it is linked with decreased physical inactivity, chronic systemic inflammation and neuropathic changes leading to motor neuron death and denervation of muscle fibres (Campbell et al. 1973; Degens 2010). However, the presence of obesity coupled with sarcopenia has

been shown to exacerbate functional limitations, increasing the difficulty in performing physical functions that require strength (Rolland et al. 2009). Baumgartner et al. (2004) defined the combination of these morbidities as 'sarcopenic obesity'. Individuals were classified as sarcopenic obese through having an appendicular skeletal muscle mass index [skeletal muscle mass (kg) ÷ stature$^2$ ($m^2$)] greater than two standard deviations below that of a 20-30 years old young adult reference group (Baumgartner et al. 1998), combined with a body fat percentage above the 60$^{th}$ percentile (Baumgartner et al. 2004).

As discussed above, obesity independent from sarcopenia, has been associated with difficulty in performing daily physical functions such as lifting heavy objects and stair negotiation. Individuals with sarcopenic obesity have an even greater difficulty in performing these daily physical functions (Rolland et al. 2009). This research was supported by Zoico *et al*. (2004) who reported older obese women to have a 3-4 times increased risk of developing functional limitations, where their BMI was higher than 30. However within this study, individuals who had class II sarcopenia (i.e. skeletal muscle mass index 2 standard deviations below a young adult reference group (Janssen et al. 2002)) had a similar risk of functional limitations as the females who were only characterised as obese. This research suggests that both conditions play a role in limiting physical performance during daily tasks, yet the interaction intensifies the unfavourable consequences of the morbidities. A rationale into the exacerbation of sarcopenia brought about by obesity is potentially due to increased mechanical stress to the musculo-skeletal system through carrying the inert mass of high levels of adipose tissue evident in obesity. In addition, adipose tissue is known to act as an endocrine organ, secreting numerous hormones and inflammatory cytokines (Ahima and Flier 2000), hence enhancing biochemical stress. Obese individuals store chronically high levels of adipose tissue, which causes an increase in circulating pro-inflammatory cytokines (Hotamisligil et al. 1995). Pro-inflammatory cytokines, such as tumour necrosis factor alpha (TNF-α) (Hotamisligil et al. 1995), interleukin -1α (Juge-Aubry et al. 2003), interleukin-6 (IL-6) (Park et al. 2005) and C-reactive protein (CRP) (Park et al. 2005) play a role in cell signalling in the response to both acute and chronic systemic inflammation and can have a detrimental impact on skeletal muscle by stimulating muscle protein degradation (Garcia-Martinez et al. 1993) causing muscle wasting/atrophy and reducing muscle protein synthesis (Mercier et al. 2002). The initiation of muscle wasting/atrophy is modulated via numerous mechanisms such as

activation of the ubiquitin-proteasome pathway (Cao et al. 2005; Degens 2010; Saini et al. 2006), which has been shown to be effected via TNF-α (Llovera et al. 1998). Chronically high levels of TNF-α initiates protein degradation and decreased protein synthesis (Mercier et al. 2002), with the net effect being skeletal muscle atrophy.

The decrease in protein synthesis can also be related to a reduction in anabolic hormones that would otherwise promote the repair and regeneration of skeletal muscle. This is observed in the reduction in Insulin-Like Growth Factor-1 (IGF-1), a promoter of protein synthesis and muscle hypertrophy (DeVol et al. 1990), as reported in severely obese women (Galli et al. 2012). However within said study, IGF-1 levels following a surgical intervention (laparoscopic adjustable gastric banding), increased proportionately to the extent of weight loss. This therefore demonstrates that lowering adiposity can improve an individual's anabolic profile. The inhibition of IGF-1 is thought to be initiated by the TNF-α-mediated activation of Jun N-terminal kinase (JNK) (Grounds et al. 2008). Activation of JNK has also been shown to play a role in the development of insulin resistance and metabolic syndrome through diet induced obesity (Sabio et al. 2010). The overall implications of low IGF-I levels coupled with elevated pro-inflammatory cytokines in an obese individual, would be a blunting of any beneficial effect of enhanced loading. Such an effect may be further exacerbated in an elderly population owing to a less than optimal endocrine milieu normally associated with normal ageing: i.e. low IGF-1, growth hormone and testosterone (Bucci et al. 2013; Lamberts et al. 1997) levels, combined with higher fat infiltration within skeletal muscle (Delmonico et al. 2009) and the 'inflamed ageing' phenomenon i.e. higher circulatory levels of pro-inflammatory cytokines (Visser et al. 2002).

In the literature on the effects of obesity on muscle function in an elderly population, Rolland *et al*. (2004) examined upper and lower limb muscle strength in obese elderly women and how the effect of habitual physical activity levels contributed to any differences in maximum muscle strength between active/non-active obese elderly individuals. The study consisted of three cohorts: (i) obese (n=215; BMI=31.9); (ii) normal weight (n=630; BMI=26.3); (iii) lean (n=598; BMI=21.6) participants (it should be noted that participants ought here to have been categorised as obese, overweight and normal weight individuals, to be more correct on the terminology). Physical activity was controlled for and defined as being active by taking part in at least one recreational physical activity (i.e. hiking,

swimming and gardening) for greater than one hour per week. The obese individuals were shown to be less physically active than both the lean and normal weight cohorts, yet when classifying participants as either sedentary or active, obese individuals with high activity levels demonstrated higher absolute isometric knee extension strength when compared to lean individuals. However, when individuals were classed as sedentary, any significant differences between cohorts were eradicated in relation to knee extension strength. Interestingly, there was no difference in handgrip or elbow extension strength between cohorts even though obese individuals had significantly larger arm muscle mass when compared to categorised normal weight and lean individuals (Rolland et al. 2004). This may be explained by the hypothesis presented earlier regarding elevated adiposity causing additional overloading of the anti-gravity muscles (e.g. quadriceps, triceps surae) during routine daily activities, e.g. walking, climbing steps, etc. This creates an environment of hypergravity, which has been shown to increase isokinetic plantar flexor strength by 40% in post-menopausal women following 12 weeks of resistance training using weighted vests (Klentrou et al. 2007).

This benefit of weighted exercise has also been shown in women aged between 50-75 years, who saw increases in muscle strength, power and lean leg mass after a nine-month training regime (Shaw and Snow 1998). In addition to these variables, the participant's postural stability was improved in a medio-lateral direction. This specific improvement in postural balance has been shown to benefit elderly frail individuals, as most falls occur in a medio-lateral plane (Greenspan et al. 1998). Contrary to this beneficial effect of weighted exercise, obese individuals are shown to have poor postural stability (Maffiuletti et al. 2005). Thus, even though comparisons may be made between the additional loading of a hypergravity environment against the excess loading experienced by an obese individual (through their own body mass), the detrimental consequences of obesity appear to outweigh any potential benefits of increased loading. However, as shown by Rolland and colleagues (2004), increasing physical activity levels may potentiate an increase in muscle strength thereby lessening the detrimental consequences of obesity.

Villarreal *et al*. (2004) looked into the association between physical frailty and body composition in obese elderly (n=52), non-obese frail elderly (n=52) and non-obese non-frail elderly (n=52). The classification of physical frailty was defined using three specific tests: a modified physical performance test (PPT) consisting of seven standardised timed tasks (such as a 50 foot walk, putting on and

removing a coat, standing up from a 16 inch chair five times and climbing a flight of stairs), Peak aerobic power ($VO_2$ peak) using a graded treadmill test and a log of their activities during daily living. From these three tests, physical frailty was then defined if participants met two out of three of the following criteria: modified PPT score of between 18-32, $VO_2$ peak of 11-18 mL/kg/min$^{-1}$ and difficulty in performing two daily activities (Villareal et al. 2004). Within the study it was reported that the obese elderly individuals had greater absolute FFM than both non-obese frail and non-obese non-frail cohorts, yet when normalised to total body mass it was found to be lower. In addition, the obese individuals had poorer muscle quality, i.e. lower knee extension strength relative to leg lean mass, compared to their non-obese counterparts. A limitation of this assertion is that DEXA, as opposed to MRI or ultrasound, cannot differentiate between muscle groups. This is important, due to the potential error in relating the torque produced to whole leg lean mass instead of the muscle group undertaking the specific task.

Delmonico *et al*. (2009) examined the effects of sarcopenic obesity on muscle strength and physical function. They reported an age-related increase in intramuscular fat content at mid-thigh in both men and women. Due to this being a longitudinal study it was reported that after 5 years, intramuscular fat content increased irrespective of changes in body mass and subcutaneous fat in the thigh. Coupled with the increase in intramuscular fat, it was reported that the loss of strength was 2-5 times greater than the loss of muscle mass with ageing (Delmonico et al. 2009). This study demonstrates that the loading effect seen in younger individuals does not attenuate the age-related loss of strength. The disproportionate loss of strength vs. muscle mass was suggested through a loss in muscle quality, which has previously been reported by Goodpaster *et al*. (2006) in an elderly cohort. These studies suggest that the rate of force loss with ageing is similar in both obese and non-obese persons. It would not be unreasonable to expect the positive association between recreational physical activity and lower limb strength in elderly obese individuals (Rolland et al. 2004) to offset a decrease in muscle quality.

In summary, with the increase in life expectancy and the rise in obesity, it is unsurprising that sarcopenic obesity incidence is also increasing (James 2008). However, there appears to be limited information regarding the effects of sarcopenic obesity on skeletal muscle structure and function. In contrast, ageing has been associated with lower agonist muscle activation (Morse et al. 2004), an increase in antagonist muscle co-activation (Klein et al. 2001), a decrease in

muscle fascicle pennation angle (Morse et al. 2005b) and lower muscle volume (Thom et al. 2005). To-date, these adaptations have not been systematically examined in an obese elderly population. Nonetheless, current data suggest that obesity exacerbates the age-related physical function limitations associated with the loss of muscle mass and strength.

## Conclusion

Obesity is recognised as being a worldwide epidemic and a major public health concern (James 2008). It has been reported to have detrimental implications for the functioning of skeletal muscle yet very little is known about the specific adaptations of skeletal muscle by gender and age, in the presence of chronically elevated adiposity.

The consensus within the literature is that obese individuals have reduced maximum muscle strength in their anti-gravity muscles relative to body mass compared to non-obese persons (Abdelmoula et al. 2012; Blimkie et al. 1990; Hulens et al. 2001; Lafortuna et al. 2005; Maffiuletti et al. 2008; Maffiuletti et al. 2007; Rolland et al. 2004; Delmonico et al. 2009). This effect on an obese individual is shown to increase the risk of developing osteoarthritis (Slemenda et al. 1998) and potentially cause functional limitations especially in the elderly (Visser et al. 2005). Evidence (in adolescent but not yet shown in adults and/or elderly persons) suggests that high levels of adiposity may impair agonist muscle activation, adding to or perhaps leading to the functional limitation of low strength relative to body mass. In addition, it is not known whether adiposity affects antagonist muscle co-activation, thus having implications for measuring maximum strength. Another gap in the literature relates to knowledge on the effect of obesity on muscle volume, PCSA and architectural measurements are required to improve its methodology as the use of overall lean mass/FFM, leg lean mass and muscle anatomical cross-sectional area, estimates the effect adiposity has on muscle structure and can therefore potentially under estimate its effect.

Therefore, future research is needed to systematically investigate whether body fat percentage *per se* may be related to agonist muscle activation and antagonist co-activation and/or morphological characteristics, such as muscle volume, PCSA and architecture in young and older adults. From such a knowledge, would aide in the potential development of therapeutic targets to be developed.

## Structure of PhD Thesis

**Chapter 2:** The effect of body composition and ageing on the neural determinants of maximal strength in both the young and old females

**Participants**

Young (18-49yrs old) : n=54 Old (50-80 yrs old) n=48

**Measures**

- Plantar flexion/ Dorsiflexion maximum voluntary torque (MVC)
- Agonist muscle activation using the interpolated twitch technique
- Antagonist co-contraction using surface electromyography
- Leg Lean mass from dual energy x-ray absorptiometry

**Exclusion criteria**

- Self-reported as habitually undertaking structured exercise for more than 3 hours per week.
- Joint pathologies that affect the participants' ability to exert and maximum torque.
- The use of non-steroidal anti-inflammatory drugs.
- Self-reported signs consistent with peripheral neuropathy (i.e. muscle weakness)

**Chapter 3:** The effect of body composition and ageing on the muscle architecture and in both the young and old females

**Participants**

Young (18-49yrs old) : n=52 Old (50-80 yrs old) n=48

**Measures**

- Gastrocnemis medialis (GM) Anatomical cross sectional area (25, 50 and 75% of muscle length)
- GM physiological cross sectional area
- GM fascicle length (at rest and during MVC)
- GM pennation angle (at rest and during MVC)
- GM muscle volume

**Exclusion criteria**

- Self-reported as habitually undertaking structured exercise for more than 3 hours per week.
- Joint pathologies that affect the participants' ability to exert and maximum torque.
- The use of non-steroidal anti-inflammatory drugs.
- Self-reported signs consistent with peripheral neuropathy (i.e. muscle weakness)

Participants were the same sample as in Chapter 2, with the exception of 2 young persons for whom muscle volume files were inadvertently lost

**Chapter 4:** The effect of body composition and ageing on intrinsic muscle strength in both the young and old females

**Participants**

Young (18-49yrs old) : n=49 Old (50-80 yrs old) n=45

**Measures**

- Intrinsic strength (MVC/GM muscle volume)
- GM Specific Force

**Exclusion criteria**

- Self-reported as habitually undertaking structured exercise for more than 3 hours per week.
- Joint pathologies that affect the participants' ability to exert and maximum torque.
- The use of non-steroidal anti-inflammatory drugs.
- Self-reported signs consistent with peripheral neuropathy (i.e. muscle weakness)

Participants were the same sample as in Chapter 2, with the exception of 5 young and 3 older persons with missing muscle volume and architecture ultrasound files

**Chapter 5:** The continuum of adiposity and its effect on skeletal muscle size, structure and function in untrained young versus old males

**Participants**

Young (18-49yrs old) : n=16 Old (50-80 yrs old) n=18

**Measures**

- MVC corrected for antagonist co-contraction and agonist muscle activation
- GM muscle volume
- Intrinsic strength (MVC/GM muscle volume)
- GM Specific Force

**Exclusion criteria**

- Self-reported as habitually undertaking structured exercise for more than 3 hours per week.
- Joint pathologies that affect the participants' ability to exert and maximum torque.
- The use of non-steroidal anti-inflammatory drugs.
- Self-reported signs consistent with peripheral neuropathy (i.e. muscle weakness)

**Chapter 6:** A between genders contrast of the impact of degree of adiposity on skeletal muscle structural and functional properties

**Participants**

Male (n=34); Female (n=94)

**Measures**

- MVC corrected for antagonist co-contraction and agonist muscle activation
- GM muscle volume
- Intrinsic strength (MVC/GM muscle volume)
- GM Specific Force

Participants were the same sample as in Chapter 4 (for females) and Chapter 5 (for males), - i.e. all those participants with a complete dataset

## Chapter 2: Combined effects of body composition and ageing on joint torque, muscle activation and co-contraction in inactive women

Data as presented in: Tomlinson DJ, Erskine RM, Morse CI, Winwood K, Onambele-Pearson GL (2014) Combined effects of body composition and ageing on joint torque, muscle activation and co-contraction in sedentary women. Age (Dordr) 36 (3):9652. doi:10.1007/s11357-014-9652-1

Data as presented in: Tomlinson DJ, Erskine RM, Morse CI, Winwood K, Onambele-Pearson GL. The Effect of Adiposity on Skeletal Muscle Size and Strength in Untrained Women. Poster session presented at the Poster session presented at the 37th Congress of the International Union of Physiological Sciences, Birmingham (2013).

## Abstract

This study aimed to establish the interplay between body mass, adiposity, ageing and determinants of skeletal muscle strength. 102 untrained healthy women categorised by age into Young (Y) (mean ± SD: 26.7 ± 9.4 yrs) versus old (O) (65.1 ± 7.2 yrs) and BMI classification (underweight, normal weight, overweight and obese). Participants were assessed for body fat and lean mass using dual energy x-ray absorptiometry, plantar flexion and dorsi flexion, maximum voluntary isometric contraction (MVC) torque, muscle activation capacity using the interpolated twitch technique and antagonist muscle co-contraction using surface electromyography. MVC torque normalised to body mass in the obese group was 35% and 29% lower ($p<0.05$) in Y and 34% and 31% lower ($p<0.05$) in the O, compared with underweight and normal weight individuals respectively. Y with ≥ 40% body fat had significantly lower activation than Y with < 40% body fat (88.3% vs. 94.4%; $p<0.05$), but O did not exhibit this effect. Co-contraction was affected by ageing (16.1% in O vs. 13.8% in Y; $p<0.05$) but not body composition. There were significant associations between markers of body composition, age, strength and activation capacity, with the strongest correlation between muscle strength and total body mass ($r^2 = 0.508$ in Y; $p<0.001$ vs. $r^2 = 0.204$ in O; $p<0.01$). Furthermore, the age related loss in PF MVC torque was exacerbated in obese compared to underweight, normal weight and overweight individuals (-0.93% vs. -0.62%, -0.69% and -0.70% per year). The negative impact of adiposity on muscle performance is associated not only with muscular, but also neural factors. Overall the effects of ageing and obesity on this system are somewhat cumulative.

## Introduction

Obesity is associated with high body fat and several co-morbidities including lowered functional mobility, particularly in the elderly (Zoico et al. 2004). The latter effect is likely linked to decreased strength:body mass ratio in obese compared with normal weight individuals in both young (Maffiuletti et al. 2007) and aged populations (Rolland et al. 2004). Contributors to strength, over and above muscle tissue content (Erskine et al. 2010a), are agonist muscle activation (Morse et al. 2004) antagonist co-contraction (Klein et al. 2001) and tendon moment arm (Erskine et al. 2010a). Yet, the link between obesity and the principal factors contributing to decreased strength:body mass ratio in both a young and ageing populations has yet to be explored. The combined impact of ageing and obesity is of particular interest, as strength is known to decline with ageing (Morse et al. 2005b) yet it remains to be seen what effect, if any, increased adiposity has on the ageing-related sarcopenia, and in particular on the neuromuscular components of muscle strength.

It could be hypothesised that obesity in both young and old cohorts would load the antigravity muscles favourably similarly to being placed in a hypergravity environment (Blimkie et al. 1990; Maffiuletti et al. 2013; Maffiuletti et al. 2007). Indeed, 3 weeks of simulated hypergravity in young athletes wearing a weighted vest ranging from 7-13% bodyweight from morning until night, increased participants' muscle power (Bosco et al. 1984; Bosco 1985; Bosco et al. 1986). Interestingly, similar increases in muscle power were demonstrated in postmenopausal women who utilised weighted vests (Klentrou et al. 2007). When relating this to an obese individual who has carried excess weight continuously for years not weeks, strength gains may follow those seen with resistance training thus having both neural and muscular underpinnings (Erskine et al. 2010b). Although the loading intensity of increased adiposity is not as high in the conventional resistance training regimes, the volume of loading is likely to be higher with lower loads being lifted during 'repetitions' of daily tasks and over longer periods of time. In support of this hypothesis, low-load high-volume resistance exercise has been shown to stimulate muscle protein synthesis more than traditional high-load, low volume exercise (Burd et al. 2010).

In terms of the muscular factors of decreased strength, muscle strength is 24.2% and 22.2% higher (absolute torque or torque normalised to thigh muscle mass, respectively) in obese compared to non-obese adolescent boys

(Abdelmoula et al. 2012). In contrast lower limb MVC torque and power (both absolute and normalised to muscle volume) is lower in obese compared to non-obese persons (Hilton et al. 2008). In terms of the neural factors of decreased strength, one limiting factor could be the central drive. However, studies report that increased adiposity is in fact associated with increased neural sympathetic drive (Alvarez et al. 2002). This would therefore suggest that the deleterious effects of weakness associated with high fat mass are muscular but not neural in origin. However, a study reported a decrease in the muscle activation capacity (85.1% vs. 95.3%) in obese compared to non-obese adolescent males (Blimkie et al. 1990). Unfortunately, exercise training status was not monitored in this study, and this could account for differences in muscle activation (Hakkinen et al. 1998). In another study, it was found that obese individuals had greater fat free mass yet similar strength values compared to their normal weight counterparts (Rolland et al. 2004), hence further supporting the suggestion of obesity-related weakness having a neural basis (e.g. decreased agonist activation capacity). Moreover, whilst the expectation is that with increased age there will be a decrease in agonist activation capacity even in the absence of obesity (Morse et al. 2004), it is unclear whether the above-described decreased activation capacity seen in the adolescents would be mirrored in adults and/or exacerbated in the elderly.

Indeed, skeletal muscle ageing has been well documented and is characterised by lower muscle strength (Morse et al. 2005b; Onambele et al. 2006a; Onambele et al. 2006b), muscle volume (Thom et al. 2005), agonist activation (Morse et al. 2004) and increased antagonist co-contraction (Klein et al. 2001). The consensus is that many of these ageing effects are caused through decreased habitual physical activity levels and sarcopenia (Rosenberg 1997). Hence, since there is a recognised age-related prevalence in increased body fat percentage termed sarcopenic obesity (Zamboni et al. 2008), characterised by a combination of reduced skeletal muscle mass and increased intramuscular fat (Hilton et al. 2008), obesity would further compound the ageing effects. In support of this hypothesis, sarcopenic obesity would be expected to aggravate the impact of ageing on physical functions including stair climbing, rising from a chair and lifting objects (Rolland et al. 2009). However, Rolland and colleagues (2004) did not quantify either agonist muscle activation capacity or antagonist co-contraction, thus potentially masking the true impact of sarcopenic obesity.

The present study therefore aimed to contrast how different levels of BMI vs. lean muscle mass vs. adiposity impact on both muscular (absolute and normalised

MVC ankle joint torque) and neural factors (agonist muscle activation, antagonist co-contraction) underlying skeletal muscle function. The study also aimed to determine whether the effects of ageing and adiposity were additive. It was hypothesised that: (1) Absolute torque in both young and old obese individuals would be higher, but torque relative to body mass lower, compared to underweight, normal weight and overweight individuals. (2) Muscle activation would be significantly lower in obese young and old individuals. (3) The slope of the relationship between adiposity and joint torque, or activation capacity, would be emphasised in the older individuals relative to their younger counterparts.

## Method

**Participants**:

Untrained females (n= 102) categorised by age into Young (Y) 18-49 years old, or old (O) 50-80 years old, volunteered to take part in this study. Participants were sub-categorised into four Body mass index classifications (BMI – Body Mass (kg)/Stature$^2$ (m)) into Underweight (BMI < 20), Normal (BMI 20-24.9), Overweight (BMI 25-29.9) and Obese (BMI > 30). Group information on age, stature, and body mass is presented in Table 2.1. Participants were excluded if there was any issue with lower limb muscles/joints affecting their mobility or ability to exert maximum force. Fasted blood glucose levels were used as an indication of undisclosed peripheral neuropathy, a condition which has a detrimental effect on force production (Hilton et al. 2008). Physical activity status was screened by the Baeke physical activity questionnaire (see appendix) and participants were excluded if they self-reported as habitually undertaking structured exercise for more than 3 hours per week. Physical activity was found not to be a confounding factor due to the similarity in physical activity scores between both young and old participants.

Ethical approval was obtained from the local ethics committee and all participants gave their written informed consent prior to undertaking any assessment.

**Body Composition Measure**

Body composition analysis (body content of fat, lean muscle and bone) was performed using a Dual Energy X-ray Absorptiometry (DEXA) scanner (Hologic

Discovery: Vertec Scientific Ltd, UK) with participants fasted for 12 hours prior to scanning. Participants were laid in a supine position throughout the 7 min scanning procedure. Segmental analysis of the whole body scan provided a quantification for lean leg mass, later used in the normalisation of the joint torque data. The Android to gynoid ratio (AG) of each participant was calculated using the Hologic APEX software (version 3.3). The android region was classified as the area between the mid-point of the lumber spine to the top of the pelvis, whilst the gynoid region was classified as the area between the head of the femur and mid thigh.

**Muscle Strength**

Plantar Flexion (PF) and Dorsi Flexion (DF) Maximum voluntary contractions (MVC) torque were assessed in the dominant limb using an isokinetic dynamometer (Cybex Norm, Cybex International, New York, NY). Participants were seated with a hip angle of 85° and their dominant leg fully extended. The dominant foot was secured to the footplate of the dynamometer using inextensible straps, ensuring the lateral malleolus was aligned with the centre of rotation. Participants were firmly strapped at the hip, distal thigh and chest with inextensible straps to minimise movement. Prior to undertaking any MVCs, the participants completed a series of warm-up PF and DF contractions.

Participants subsequently performed four isometric PF (×2) and DF (×2) MVCs with the ankle positioned at 0° (anatomically neutral), with 1-2 min rest between efforts. MVCs were repeated if there was > 10% difference between MVCs to ensure true MVC was obtained. The highest recorded PF and DF MVCs were used for subsequent analysis. Surface electromyography (EMG) of the tibialis anterior (TA) was recorded during all MVCs to calculate antagonist muscle co-contraction during PF MVC (see below for details). Verbal encouragement and biofeedback were provided during each effort.

**Antagonist Muscle Co-contraction**

Surface Electromyography (EMG, using pre-gelled unipolar Ag-AgCl electrodes (Medicost, Denmark)) was used to assess muscle co-contraction of the tibialis anterior during PF MVC. Two electrodes (45mm x 22mm) were placed proximally at one third of the tibialis anterior muscle length, mid muscle belly with a

5mm gap separating each electrode. A reference electrode (Medicost, Denmark) was placed on the head of the fibula. Raw EMG was then recorded at 2000Hz, with band pass filter set at 10-500Hz, and notch at 50 Hz.

Muscle co-contraction (%) was calculated at 0° ankle angle utilising the raw EMG signal (computed as root mean square (RMS) 500 milliseconds either side of instantaneous peak torque) of the tibialis anterior during PF MVC divided by EMG during DF MVC. Co-contraction torque (Nm) was the product of % co-contraction and maximal DF torque.

Hence, Net PF MVC torque was calculated as the sum of observed maximal PF torque, and co-contraction torque. This method assumes that the DF EMG/Torque relationship is linear (Maganaris et al. 1998).

**Muscle Activation**

PF agonist muscle activation was estimated using the interpolated twitch technique (Morse et al. 2004; Pearson and Onambele 2006). Briefly, percutaneous stimuli (DSVDigitimer Stimulator; Digitimer, Herts., UK) were applied to the gastrocnemius using rubber stimulation pads (50mm x 100mm; American Imex; Irvin, CA, USA). The two stimulation pads were placed transversely distal to the popliteal crease and myotendinous junction of the soleus. The amplitude of the stimuli was determined prior to interpolation whilst the participant was in a relaxed state; administering twitches starting from 50 mA, with subsequent increments of 50-100 mA, until no further increase in twitch torque was elicited. The assessed supramaximal doublets (i.e. the stimulus intensity above which no further increase in twitch torque was observed with increased stimulus intensity) were superimposed during a maximal PF MVC. The calculation used to establish muscle activation is shown below:

1-(Superimposed torque increase/resting superimposed torque) × 100 = muscle activation (%)

**Statistical Analyses**

Statistical analyses were carried out using SPSS (Version 19, SPSS Inc., Chicago Illinois). To determine parametricity, Kolmogorov-Smirnov or Shapiro-Wilk (normal distribution) and Levene's tests (homogeneity of variance) were utilised. If

parametric assumptions were met, a factorial 2 × 4 ANOVA (Age × BMI) was utilised with post hoc bonferroni correction for pairwise comparisons. Where parametric assumptions were breached, Mann Whitney U or Kruskal-Wallis H were utilised. Pearson correlations described the relationships between PF MVC and leg lean mass, muscle activation, body mass, fat mass, total lean mass, body fat %, BMI and AG ratio. Additionally, linear and multiple regressions were used to determine the best predictors of PF MVC. Comparison of the regression coefficients and slopes were conducted using z-transformations and the Student's *t*-statistic. Data are reported as mean ± SD and statistical significance was accepted when $p \leq 0.05$. Study power ($\beta$) and effect size ($p\varepsilon^2$) are also reported.

## Results

### Body Composition

Table 2.1 displays the descriptive values for age, height, body mass, BMI, body fat%, total body fat, total lean mass, leg fat mass and A/G ratio for Y and O females categorised by BMI. A 2 × 4 factorial ANOVA of body fat% revealed a main effect for age ($p=0.001$; $p\varepsilon^2= 0.104$; $\beta=0.904$), for BMI ($p<0.001$; $p\varepsilon^2=0.736$; $\beta=1.000$) and an age × BMI interaction ($p=0.029$; $p\varepsilon^2=0.091$; $\beta=0.713$).

A Mann Whitney test on leg lean mass revealed a main effect of age ($p<0.001$), whilst a Kruskal-Wallis test revealed between group differences for leg lean mass between classifications in Y ($p<0.001$) and O ($p<0.001$). However, Y obese had 28% and 27% more leg lean mass than Y underweight ($p<0.001$) and Y normal weight ($p<0.001$) individuals respectively, whilst obese O had 26%, 27% and 20% more leg lean mass than O underweight ($p=0.018$), O normal weight ($p<0.001$) and O overweight ($p=0.04$), respectively (Table 2.2).

There were strong positive correlations ($p<0.001$) between leg lean mass and body mass, lean mass and body fat in both Y and O groups (Tables 2.3 and 2.4). Ageing affected neither the degree of association in these correlations ($p>0.05$) nor the slope of the regressions ($p<0.05$, Table 2.4). In predicting leg lean mass a stepwise multiple linear regression was conducted with variables body fat%, body mass, lean mass, fat mass and android/gynoid ratio in all individuals. Total lean mass and body fat% were the only predictors in a stable model, which in combination, explained 93% of the leg lean mass in both Y and O individuals ($p<0.001$; $r=0.966$).

**Table 2.1.** Descriptive variables for BMI classifications in both young and old age classifications. The AG ratio (>1.00) is utilised as an indicator for an increased risk of developing cardiovascular disease (Folsom et al. 2000). Data are presented as Mean ± SD.

| **Young (18-49)** | Underweight (n=13) | Normal (n=13) | Overweight (n=10) | Obese (n=18) | BMI Effect | Ageing Effect |
|---|---|---|---|---|---|---|
| **Age (yrs)** | 23.0 ±6.7 | 23.2 ±7.9 | 23.6 ±8.0 | 30.9 ±10.7 | p=0.002 | p=0.001 |
| **Height (cm)** | 167.5 ±4.7 | 164.1 ±8.6 | 162.8 ±7.4 | 166.5 ±7.6 | p=0.422 | p=0.002 |
| **Body Mass (kg)** | 52.7 ±3.9 | 58.3 ±6.5 | 74.6 ±8.3 | 97.5 ±13.1 | p<0.001 | p=0.683 |
| **BMI (kg/m$^2$)** | 18.8 ±0.9 | 21.6 ±1.1 | 28.1 ±2.4 | 35.2 ±4.4 | p<0.001 | p=0.625 |
| **Body Fat (%)** | 26.5 ±3.9 | 30.4 ±3.5 | 38.7 ±5.9 | 45.3 ±3.9 | p<0.001 | p=0.001 |
| **Total Body Fat (kg)** | 13.7 ±2.2 | 17.2 ±2.7 | 28.5 ±6.8 | 43.2 ±7.3 | p<0.001 | p=0.376 |
| **Total Lean Mass (kg)** | 35.7 ±3.4 | 37.2 ±4.7 | 42.0 ±4.3 | 49.4 ±7.0 | p<0.001 | p=0.002 |
| **Fat Mass Leg (kg)** | 3.2 ±0.5 | 3.8 ±0.6 | 5.8 ±1.8 | 7.7 ±1.5 | p<0.001 | p=0.859 |
| **A/G Ratio** | 0.70 ±0.09 | 0.77 ±0.12 | 0.95 ±0.14 | 1.06 ±0.08 | p<0.001 | p=0.062 |

| **Old (50-80)** | Underweight (n=4) | Normal (n=15) | Overweight (n=18) | Obese (n=11) | BMI Effect | Ageing Effect |
|---|---|---|---|---|---|---|
| **Age (yrs)** | 63.8 ±5.7 | 63.5 ±7.7 | 68.2 ±4.8 | 62.5 ±9.0 | p=0.183 | p=0.001 |
| **Height (cm)** | 159.1 ±5.3 | 159.4 ±5.2 | 162.1 ±3.8 | 162.5 ±5.7 | p=0.264 | p=0.002 |
| **Body Mass (kg)** | 48.4 ±4.2 | 56.6 ±4.3 | 71.6 ±4.5 | 90.1 ±16.4 | p<0.001 | p=0.683 |
| **BMI (kg/m$^2$)** | 19.1 ±0.8 | 22.2 ±1.0 | 27.3 ±1.2 | 34.1 ±5.7 | p<0.001 | p=0.625 |
| **Body Fat (%)** | 26.5 ±2.1 | 36.0 ±3.6 | 42.9 ±3.3 | 46.1 ±5.0 | p<0.001 | p=0.001 |
| **Total Body Fat (kg)** | 12.5 ±2.0 | 19.9 ±2.9 | 29.8 ±3.4 | 40.9 ±11.3 | p<0.001 | p=0.376 |
| **Total Lean Mass (kg)** | 32.8 ±2.4 | 33.3 ±2.4 | 37.4 ±2.4 | 44.7 ±6.7 | p<0.001 | p=0.002 |
| **Fat Mass Leg (kg)** | 2.8 ±0.3 | 3.8 ±0.7 | 5.6 ±0.1 | 6.5 ±2.0 | p<0.001 | p=0.859 |
| **A/G Ratio** | 0.66 ±0.09 | 0.89 ±0.16 | 0.97 ±0.11 | 1.10 ±0.08 | p<0.001 | p=0.062 |

**Muscle Strength**

A 2 × 4 Factorial ANOVA on PF MVC torque revealed a main effect for age ($p<0.001$; $p\varepsilon^2= 0.327$; $\beta=1.000$), a main effect for BMI ($p=0.001$; $p\varepsilon^2=0.152$; $\beta=0.937$), yet there was no significant age × BMI interaction ($p=0.676$; $p\varepsilon^2=0.016$; $\beta=0.151$). However Y obese had 23% and 20% higher uncorrected PF MVC torque than underweight ($p<0.008$) and normal weight ($p=0.039$) individuals, whilst O individuals revealed no significant between group differences ($p>0.05$) (Table 2.2).

PF MVC torque relative to body mass was higher in Y vs. O ($p<0.001$; $p\varepsilon^2= 0.291$; $\beta=1.000$), and differed according to BMI ($p<0.001$; $p\varepsilon^2=0.285$; $\beta=1.000$), but there was no significant age × BMI interaction ($p=0.410$; $p\varepsilon^2=0.030$; $\beta=0.258$). However Y obese had 43%, 35% and 25% lower PF MVC torque relative to body mass than Y underweight ($p<0.001$), Y normal weight ($p<0.001$) and Y overweight ($p=0.031$) respectively, whilst obese O exhibited 43% lower uncorrected PF MVC torque relative to body mass than O underweight ($p=0.032$) (Table 2.2).

Net PF MVC torque revealed a main effect of age ($p<0.001$; $p\varepsilon^2= 0.293$; $\beta=1.000$) and BMI ($p<0.001$; $p\varepsilon^2=0.224$; $\beta=0.995$), but no significant age × BMI interaction ($p=0.581$; $p\varepsilon^2=0.021$; $\beta=0.184$). However Y obese had 27% and 23% higher corrected PF MVC torque than Y underweight ($p<0.001$) and Y normal weight ($p=0.002$) individuals, whilst O revealed no significant between group differences ($p>0.05$) (Table 2.2).

Net PF MVC torque/body mass revealed a main effect of age ($p<0.001$; $p\varepsilon^2= 0.281$; $\beta=1.000$) and BMI ($p<0.001$; $p\varepsilon^2=0.280$; $\beta=1.000$), but no significant age × BMI interaction ($p=0.821$; $p\varepsilon^2=0.010$; $\beta=0.107$). However Y obese had 35% and 29% lower net PF MVC torque/body mass than Y underweight ($p<0.001$) and Y normal weight ($p<0.001$) individuals, whilst obese O had 31% lower net PF MVC torque/body mass than O normal weight ($p=0.016$) (Table 2.2).

There were strong positive correlations ($p<0.001$) between Net PF MVC torque and body mass, lean mass and body fat in both Y and O groups (as seen in Table 2.3). Ageing affected neither the degree of association in these correlations ($p>0.05$) nor the slope of the regressions ($p>0.05$, Table 2.4). In predicting net PF MVC a stepwise multiple linear regression was conducted with independent variables being body fat%, body mass, lean mass, fat mass and android/gynoid ratio, for the pooled study population (i.e. all Y and O data). Total lean mass was

the only predictor in the stable model, and explained 51% of net PF MVC and body mass normalised torque regardless of age ($p<0.001$; $r=0.710$).

Net PF MVC and leg lean mass were correlated in both the Y ($p<0.001$; $r^2 = 0.623$) and O ($p<0.001$; $r^2 = 0.260$) age groups, with similar slopes in the two age groups (Table 2.4).

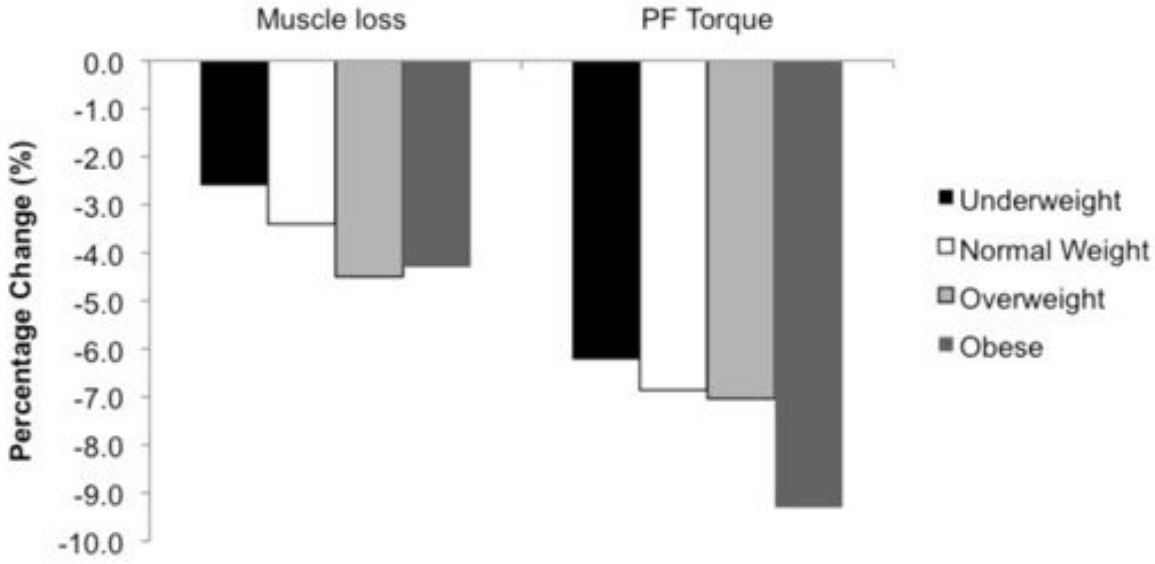
Figure 2.1 displays the % change per 10 years (assuming % change is linear) in muscle loss and PF torque categorised by BMI.

**Table 2.2.** Displays strength data (PF torque/net PF torque corrected for agonist muscle activation and antagonist co-contraction), agonist activation capacity, antagonist co-contraction and leg lean mass in both young and old BMI classifications. Data are presented as Mean ± SD.

| | Young | | | | Old | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | U (n=13) | N (n=13) | O (n=10) | Ob (n=18) | U (n=4) | N (n=15) | O (n=18) | Ob (n=11) | Young BMI effect | Old BMI effect | Ageing Effect |
| **PF Torque** | | | | | | | | | | | |
| **PF MVC (Nm)** | 125.7 ±22.3 | 131.6 ±28.1 | 150.6 ±34.8 | 163.7 ±27.7 | 94.4 ±32.6 | 95.5 ±17.1 | 102.9 ±27.0 | 118.0 ±30.2 | U N / Ob | - | p<0.001 |
| **MVC Relative to body weight (Nm/kg)** | 2.38 ±0.38 | 2.26 ±0.45 | 2.09 ±0.34 | 1.67 ±0.30 | 1.96 ±0.67 | 1.69 ±0.28 | 1.44 ±0.35 | 1.33 ±0.37 | UN O /Ob | U / Ob | p<0.001 |
| **Net PF Torque** | | | | | | | | | | | |
| **PF MVC (Nm)** | 134.9 ±21.4 | 142.1 ±25.7 | 165.8 ±33.3 | 185.4 ±36.8 | 105.6 ±28.1 | 109.6 ±17.4 | 122.6 ±26.9 | 134.7 ±31.9 | U N / Ob | - | p<0.001 |
| **MVC Relative to body weight (Nm/kg)** | 2.56 ±0.38 | 2.45 ±0.41 | 2.27 ±0.27 | 1.90 ±0.30 | 2.03 ±0.40 | 1.99 ±0.39 | 1.71 ±0.35 | 1.52 ±0.39 | U N /Ob | N / Ob | p<0.001 |
| **DF MVC (Nm)** | 22.8 ±7.2 | 25.3 ±5.0 | 27.0 ±7.2 | 26.9 ±7.0 | 20.1 ±4.1 | 19.6 ±3.9 | 22.6 ±5.0 | 28.4 ±6.3 | - | U N O /Ob | p=0.025 |
| **Activation (%)** | 95.0 ±5.0 | 93.9 ±7.8 | 94.1 ±6.3 | 87.9 ±10.4 | 90.1 ±12.1 | 86.4 ±10.6 | 82.2 ±13.7 | 84.0 ±15.2 | - | - | p=0.001 |
| **Co-contraction (%)** | 15.7 ±7.2 | 17.2 ±8.5 | 15.2 ±5.8 | 16.1 ±5.9 | 15.9 ±10.6 | 15.1 ±8.8 | 12.9 ±7.9 | 12.8 ±6.8 | - | - | p=0.042 |
| **Leg Lean Mass (kg)** | 5.99 ±0.78 | 6.10 ±0.97 | 7.31 ±1.05 | 8.30 ±1.35 | 5.37 ±0.52 | 5.26 ±0.52 | 5.81 ±0.64 | 7.21 ±1.38 | UN / Ob | U N O /Ob | p<0.001 |

(U= underweight, N = normal weight, O = overweight, Ob = obese

**Figure 2.1**. Relative change (mean % change per 10 years assuming percentage change is linear) by BMI class (a) muscle loss and b) PF torque.




**Muscle Co-contraction**

There was no significant difference in antagonist co-contraction between BMI groups (p=0.730) (Table 2.2). Yet, there was an effect of age, with O exhibiting lower co-activation than Y (16.1 vs. 13.8%; p=0.042).

**Table 2.3.** Linear Regressions ($r^2$) between net PF torque, leg lean mass and agonist muscle activation against a series of descriptive variables in young and old untrained females (* P<0.05, ** P<0.01, *** P<0.001).

| | Young (n=54) | | | Old (n=48) | | |
|---|---|---|---|---|---|---|
| | PF MVC | Leg lean Mass | Activation | PF MVC | Leg lean Mass | Activation |
| Leg lean mass | 0.623*** | - | 0.179** | 0.26*** | - | NS |
| Body mass | 0.508*** | 0.749*** | 0.164** | 0.204** | 0.677*** | NS |
| Fat mass | 0.385*** | 0.538*** | 0.157** | 0.135* | 0.472*** | NS |
| Lean mass | 0.600*** | 0.936*** | 0.125** | 0.242*** | 0.904*** | NS |
| Body fat % | 0.203** | 0.240*** | 0.103* | NS | 0.135* | NS |
| BMI | 0.411*** | 0.548*** | 0.179** | 0.157** | 0.559*** | NS |

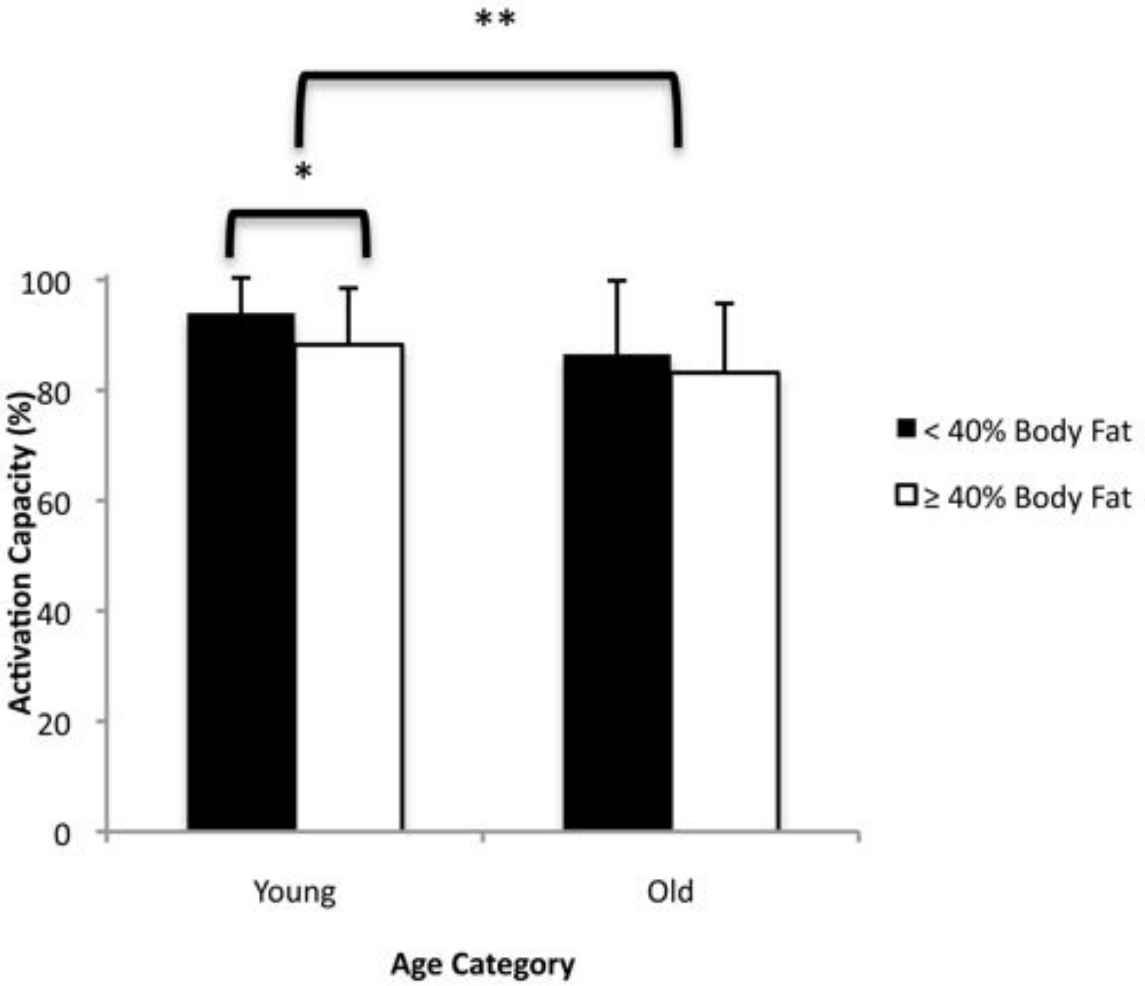
**Table 2.4.** Pearson correlations, z-transformation of r and Student's *t*-statistic between net PF MVC and leg lean mass against a series of descriptive variables in young and old untrained females (* P<0.05, ** P<0.01, *** P<0.001) (If Z > 1.96, p<0.05; Z > 2.58, p<0.01) (Student's *t*-statistic significance if *t* falls outside ± 1.96 p<0.05)

| | Young | | | Old | | | Correlation co-efficient | Ageing Effect |
|---|---|---|---|---|---|---|---|---|
| | n | r value | slope | n | r value | slope | Z-transformation of r | Student's *t*-statistic |
| PF MVC vs Leg lean mass | 54 | 0.79*** | 19.63Nm/kg | 48 | 0.51*** | 12.48Nm/kg | 1.95 | 1.90 |
| PF MVC vs Body mass | 54 | 0.71*** | 1.26Nm/kg | 48 | 0.45** | 0.75Nm/kg | 1.70 | 1.85 |
| PF MVC vs Fat mass | 54 | 0.62*** | 1.70Nm/kg | 48 | 0.37* | 0.92Nm/kg | 1.57 | 1.70 |
| PF MVC vs Lean mass | 54 | 0.78*** | 3.70Nm/kg | 48 | 0.49*** | 2.28Nm/kg | 1.93 | 1.94 |
| Leg FFM vs BM | 54 | 0.87*** | 0.06kg/kg | 48 | 0.83*** | 0.06kg/kg | 0.45 | 0.77 |
| Leg FFM vs FM | 54 | 0.73*** | 0.08kg/kg | 48 | 0.69*** | 0.07kg/kg | 0.35 | 0.74 |
| Leg FFM vs Lean mass | 54 | 0.97*** | 0.19kg/kg | 48 | 0.95*** | 0.18kg/kg | 0.50 | 0.44 |

## Voluntary Muscle Activation

Muscle activation did not differ between BMI group in either Y (p=0.138) or O (p=0.701) (Table 2.2). When Y and O were categorised by body fat % (Figure 2.2), muscle activation was higher in Y low body fat % than Y high body fat % (p=0.019) but there was no difference in O (p=0.458) (Figure 2.2). Y also demonstrated higher muscle activation capacity than O (92% vs. 84.5 %; p=0.001).

**Figure 2.2.** Impact of ageing on PF activation capacity in low body fat (<40%) and high body fat (>40%) individuals. The threshold set at ≥ 40% body fat as being defined obese, is due to previous work in an adult population (Rolland et al. 2004). Data are presented as Mean ± SD (* P<0.05; **P<0.01).




## Discussion

This data has partially confirmed the hypothesis that absolute torque in young obese individuals would be higher compared to that of underweight and normal weight individuals. However, the Old obese females did not exhibit significantly higher absolute torque compared against the other three BMI classifications. Unexpectedly, when torque was normalised to body mass, the young obese were significantly weaker than the other three BMI classifications, whereas the old obese were only weaker than their old underweight counterparts, thereby suggesting a somewhat protective impact of obesity for the old, or at least, this suggest that the effects of ageing and obesity are not necessarily cumulative

where absolute joint torque is concerned. Another partially supported hypothesis was also the observation that high adiposity only decreased activation capacity in the young whereas it had no effect in the old individuals. The summation of a negative impact of obesity to ageing in this population, was seen in terms of neuro-muscular ageing whereby there were marked differences in the slopes of the relationships between adiposity, muscle torque and activation capacity. The above observations culminated in the fact that with PF MVC torque normalised for body mass and corrected for activation and co-activation, the obesity-induced weakness was true for all pairwise BMI categories comparisons in the young. In the old however, once PF MVC torque was normalised for body mass and corrected for activation and co-activation, the only significant BMI pairwise difference was between obese vs. normal weight.

**Body Composition**

The loading effect of chronically high levels of adiposity, body mass and lean mass appear to provide a stimulus to the antigravity muscles of the lower limb similar to resistance training of increased muscle mass (Erskine et al. 2010b) (as seen in Table 2.2, 2.3 and 2.4). However whilst 3 weeks of simulated hypergravity (Bosco 1985) would cause adaptations at a neurogenic level, the mean duration of obesity is longitudinal at 13 years (Abdullah et al. 2011) and hence adaptations to this condition were not expected to be neural led. At the muscle level, low-load, high volume resistance exercise has been shown to stimulate muscle protein synthesis more than traditional high-load, low volume resistance exercise (Burd et al. 2010) and the hypothesis was that obesity would have a similar effect, thus leading to increased muscle mass and strength over time.

Leg lean mass decreased with age in all BMI classifications, but this age-related decrease was exacerbated in the overweight and obese individuals who lost 4.5% and 4.3% respectively compared to 2.6% and 3.4% in underweight and normal weight individuals every 10 years (data calculated assuming a linear regression, Figure 2.1). This blunted effect of loading in the Old participants on leg lean mass may possibly be attributed to lower levels of anabolic circulating hormones such as insulin like growth factor-1 (IGF-I) (Galli et al. 2012) and the increase of catabolic hormones such as interlukin-6 (IL-6) and tumour necrosis factor α (TNF-α) (Visser et al. 2002; Hotamisligil et al. 1995) seen in older versus younger individuals.

**Muscle strength**

Increased adiposity in the young was previously shown to have a positive effect on the loaded antigravity muscles of the knee extensors both in terms of absolute MVC isometric torque (Hulens et al. 2001; Maffiuletti et al. 2007; Abdelmoula et al. 2012) and isokinetic power (Maffiuletti et al. 2007; Hulens et al. 2002), but not when torque was made relative to body mass, as obese individuals were shown to be weaker (Maffiuletti et al. 2007). Our current results in the plantar flexors mirror these observations.

In the Old individuals there was also a trend of increasing absolute torque with BMI classification, yet there were no significant differences in MVC torque normalised to body mass between BMI classifications (Table 2.2), except, where Old obese were significantly weaker compared to normal weight individuals. This finding partly supports those of Rolland *et al*. (2004) who reported greater absolute knee extensor strength in the obese. Additionally, the Old obese individuals lost more MVC torque compared to the losses in leg lean mass (27% vs. 13%), this is comparable to research by Delmonico *et al*. (2009) who attributed losses in strength to lowering in muscle quality. This may possibly be attributed to intrinsic changes in muscle properties such as selected atrophy of type II fibres (Lexell and Taylor 1991), and/or a decrease in muscle fascicle pennation angle (Morse et al. 2005b) normally seen in ageing.

Whilst our Old females did not meet the criterion for sarcopenia (i.e. appendicular skeletal muscle mass ÷ height ($m^2$) = mean ± SD: 9.6 ± 1.5 kg/$m^2$ in this group, vs. ≤5.67 kg/$m^2$ criterion (Newman et al. 2003)), they displayed typical ageing-related characteristics compared to the young of significantly lower isometric torque (-25%, $p<0.001$), agonist activation (92.1 vs 84.5%, $p<0.05$) and leg lean mass (7.03 vs. 5.92kg, $p<0.001$). Ageing-associated α-motorneuron degeneration and denervation (Brown 1972), higher levels of IL-6 and TNF-α (Visser et al. 2002) may explain some of these effects. Assuming the loss of joint torque is linear, PF MVC torque appeared to be exacerbated in obese individuals who would lose 9.3% of maximal torque compared to 6.2%, 6.9% and 7.0% in underweight, normal weight and overweight individuals every 10 years (data calculated assuming a linear regression, Figure 2.1).

The effect of adiposity per se on muscle strength suggested a positive association with absolute PF MVC in both young and old groups (Table 2.3). Yet

when factoring total adiposity in a multiple linear regression, lean muscle mass was the only significant predictor of PF MVC torque. Since adiposity is not a contractile tissue, the apparent positive association with muscle strength can only be due to high adiposity being associated with greater muscle mass in our population.

Previous research on obesity in ageing and muscle performance, mainly selected the loaded knee extensors as an indicator of lower limb contractile capacity. The work presented in this paper is novel in that it utilised the plantar flexors, a muscle group which has a key functional relevance: a) to older persons due its documented contribution in the maintenance of postural balance (Onambele et al. 2006a), so much so that approximately 80% of the variance in postural balance can be attributed to the functional characteristics of this muscle group; b) in addition, obesity also has been documented as having a detrimental impact on postural balance (Maffiuletti et al. 2005).

**Co-contraction**

To my knowledge this is the first study to describe antagonist muscle co-contraction in an obese population. This study reports, at face value, a protective effect of obesity during ageing since Old obese individuals had significantly lower co-contraction than their younger counterparts (16.1 vs. 13.8%, $p<0.042$), thereby potentially contributing to higher agonistic forces and hence better control of joint motion. This was an unexpected finding as ageing is associated with increased co-contraction in the hamstrings (Macaluso et al. 2002), and the triceps surae (Onambele et al. 2006a). However, since joint stabilisation through co-contraction is a strategy used when muscle weakness is present (Hortobagyi and DeVita 2000), the Old obese are in fact doubly disadvantaged through being both weaker and less able to co-contract their antagonist muscles compared to age-matched normal weight counterparts, potentially leading to increased risk of joint pathologies (Felson 1995).

**Voluntary Muscle Activation**

Previous work examining muscle strength differences between obese and non-obese individuals did not correct for agonist activation and antagonist co-contraction thus underestimating the true contractile torque potential, even when

such work normalised MVC torque by muscle mass (Abdelmoula et al. 2012; Maffiuletti et al. 2007). Our study demonstrates that high levels of adiposity, with threshold set at ≥ 40% body fat (Rolland et al. 2009), has a significantly negative impact on agonist muscle activation in Y (88 vs. 94 %) but not O (83 vs. 87%) individuals. The Y data supports the Blimkie *et al*. (1990) study that reported the agonist activation capacity of obese adolescents aged 15-18 years old to be significantly lower than that of non-obese counterparts (85 vs. 95%). It would therefore appear that unlike simulated hypergravity which enhances muscle activation (Bosco et al. 1986), obesity-mediated chronic overloading has either no (as seen in the O) or a negative (as seen in the Y) impact on voluntary muscle activation. This would tend to refute the suggestion by Bosco *et al.* (1986) of a neurogenic mechanism for increased muscle power in the obese. We would argue therefore that any strengthening effect of obesity would be through muscular factors such as increased protein synthesis rate (Burd et al. 2010), which is evidenced through the phenotypic expression of greater absolute lean mass in this population.

A limitation of the present study, as with previous work (Blimkie et al. 1990; Maffiuletti et al. 2007; Abdelmoula et al. 2012), is the failure to quantify anatomical cross sectional area, and using instead leg lean mass and estimations of fat free mass and thigh muscle mass, as an indication of agonist muscle size. Not accounting for muscle fascicle pennation angle (and hence sarcomeres in parallel) and fascicle length (and hence sarcomeres in series), limits the ability to explain differences in force and power. Future work should determine a more accurate index of muscle size through computing the physiological cross sectional area (muscle volume/fascicle length) (Fukunaga et al. 1996).

**Conclusion**

The present study demonstrates that antigravity muscles adapt to chronically different levels of adiposity in both young and elderly individuals. Interestingly, the magnitude of the effect of obesity in terms of both absolute MVC joint torque and torque normalised for leg lean mass appears to be blunted in the older group. Also notable, the rate of ageing (i.e. the slope of deleterious changes in neuro-muscular properties) in the BMI sub-categories is most dramatic for the high adiposity groups.

## Chapter 3: The impact of obesity on skeletal muscle architecture in untrained young versus old women

Data as presented in: Tomlinson DJ, Erskine RM, Winwood K, Morse CI, Onambele GL (2014) The impact of obesity on skeletal muscle architecture in untrained young vs. old women. J Anat 225 (6):675-684. doi:10.1111/joa.12248

## Abstract

It is unknown if loading of the lower limbs through additional storage of fat mass as evident in obesity would promote muscular adaptations similar to those seen with resistance exercise. It is also unclear whether ageing would modulate any such adjustments. This study aimed to examine the relationships between adiposity, ageing and skeletal muscle size and architecture. 100 untrained healthy women were categorised by age into young (Y) (mean ± SD: 26.7 ± 9.4 yrs) versus old (O) (65.1 ± 7.2 yrs) and BMI classification (underweight, normal weight, overweight and obese). Participants were assessed for body fat using dual energy x-ray absorptiometry, and for gastrocnemius medialis (GM) muscle architecture (skeletal muscle fascicle pennation angle and length) and size (GM muscle volume and physiological cross sectional area (PCSA)) using B-mode ultrasonography. GM fascicle pennation angle (FPA) in the obese Y females was 25 per cent greater than underweight ($p=0.001$) and 25 per cent greater than normal weight ($p=0.001$) individuals, whilst O obese females had 32 per cent and 22 per cent greater FPA than their underweight ($p=0.008$) and normal weight ($p=0.003$) counterparts. Furthermore, FPA correlated with body mass in both Y and O females (Y $r=0.303$; $p<0.001$; O $r=0.223$; $p=0.001$), yet no age-related differences in the slope or r-values were observed ($P>0.05$). Both GM muscle volume ($p=0.003$) and PCSA ($p=0.004$) exhibited significant age × BMI interactions. In addition, muscle volume and PCSA correlated with BMI, body mass and fat mass. Interestingly, ageing reduced both the degree of association in these correlations ($p<0.05$) and the slope of the regressions ($p<0.05$).

These findings partly support the hypotheses in that obesity-associated changes in GM PCSA and volume differed between the young and old. The younger GM muscle adapted to the loading induced by high levels of body mass, adiposity and BMI by increasing its volume and increasing its pennation angle, ultimately enabling it to produce higher maximum torque. Such an adaptation to increased loading did not occur in the older GM muscle. Nonetheless, the older GM muscle increases in FPA to an extent similar to that seen in young GM muscle, an effect which partly explains the relatively enhanced absolute maximum torque observed in obese older females.

## Introduction

Obesity in both young and old individuals has been shown to induce a loading effect on skeletal muscles of the lower limbs (Lafortuna et al. 2013), increasing absolute maximal voluntary contraction (MVC) torque in obese compared to both normal and underweight individuals (Maffiuletti et al. 2007; Rolland et al. 2004). A plausible explanation for higher absolute strength may be attributed to greater fat free mass (FFM) seen in obese individuals (Maffiuletti et al. 2007). However, no previous study has quantified physiological cross sectional area (PCSA) or muscle architectural components differences in the pennate anti-gravity muscles of the lower limb in obese and non-obese individuals. This is key since PCSA, more than FFM, allows for the identification of intrinsic muscle quality (strength per unit of PCSA) differences, where fascicle length and pennation angle (i.e. architecture) effects are highlighted.

The potential impact of using muscle specific PCSA measures rather than whole limb estimates of FFM may explain the apparent discrepancy within the literature on the currently reported impact of obesity on muscle mass. Blimkie *et al*. (1990) reported no difference between obese and non-obese adolescents in quadriceps anatomical cross sectional area (ACSA) using CT. This was reiterated by Abdelmoula *et al*. (2012) from estimated thigh muscle mass using DEXA. However, in contrast Maffiuletti *et al*. (2007) reported 18% greater fat free mass in obese adults using bioelectrical impedance, whereas previous authors (Rolland et al. 2004) reported similarly increased leg muscle mass using DEXA in an elderly obese population. PCSA is directly proportional to the maximum force generated by skeletal muscle (Lieber and Friden 2000; Maganaris et al. 2001). Therefore using PCSA as a measure of muscle size would improve data comparison accuracy over ACSA and/or estimations of lean mass as utilised in previous studies, as highlighted in the paragraph above. Indeed ACSA and lean mass estimates would potentially underestimate PCSA (volume/fascicle length) (Alexander and Vernon 1975), thereby leading to an inaccurate estimation of intrinsic skeletal muscle quality.

Ageing and specifically sarcopenia, is characterised by reduced muscle PCSA, and fascicle pennation angle and length (Morse et al. 2005a). Slowing down the effects of ageing on skeletal muscle is achievable through resistance training and sustained hypergravity (Brown et al. 1990; Ferri et al. 2003; Klentrou et al. 2007; Morse et al. 2007; Reeves et al. 2004b). In contrast to the benefits of

resistance exercise or simulated hypergravity, excess adiposity does not appear to be enough of a loading stimulus to mitigate the detrimental functional consequences of obesity in the elderly (e.g. difficulties in walking, climbing stairs and rising from a chair; (Rolland et al. 2009)). Additionally a condition that has shown to exacerbate functional limitations is known as “sarcopenic obesity” which is characterised by the age related loss of muscle mass and strength plus greater intramuscular fat infiltration (Baumgartner 2000). These increases in fat infiltration coupled with sarcopenia in the elderly are reported to lead to higher levels of pro-inflammatory cytokines associated with muscle catabolism (Schrager et al. 2007), and hence potentially greater prevalence of decreased skeletal muscle mass.

To date, no study has examined the combined effect of sarcopenia and obesity in the elderly, on muscle architecture. This is a patently important area of study, as a further increased loss of sarcomeres in parallel in the obese, would detrimentally affect maximal torque production, thus highlighting the need to target this population for specific counter-measures.

The primary aim of the present study was to examine the degree of any association between BMI (or adiposity *per se,* i.e. irrespective of BMI status) and muscle architecture (fascicle length and pennation angle), as well as PCSA. A second aim was to determine whether the effects of ageing and adiposity (i.e. continued adiposity from younger to older age) were additive on these variables. It was hypothesised that: (1) muscle PCSA in both obese young and old would be greater when compared to lean, normal weight and overweight individuals. (2) Muscle fascicle pennation angle and length in obese young and old would be greater when compared to lean, normal weight and overweight individuals. (3) The slope of the relationship between adiposity, BMI, or body mass against PCSA, muscle volume, or architecture, would be lower in the older individuals compared to their younger counterparts, denoting a faster rate of changes with increased ageing.

## Method

**Participants**:

A total of 100 untrained females volunteered to take part in this study and were categorised by age into either Young (Y) 18-49 years old or Old (O) 50-80 years old (Table 3.1). Participants were then sub-categorised into four body mass index classifications (BMI – Body Mass (kg)/Stature$^2$ (m)) into Underweight (BMI < 20), Normal (BMI 20-24.9), Overweight (BMI 25-29.9) and Obese (BMI > 30). The principal exclusion criteria were issues with lower limb muscles/joints affecting mobility or ability to exert maximum torque. It should be noted here that use of non-steroidal anti-inflammatory drugs was also an exclusion criterion. In addition, whilst three study participants had controlled type II diabetes mellitus, they did not in fact display any characteristics of peripheral neuropathy, such as motor dysfunction and weakness. Physical activity status was screened by the Baeke physical activity questionnaire (see appendix) and participants were excluded if they self-reported as habitually undertaking structured exercise for more than 3 hours per week. Physical activity was found not to be a confounding factor due to the similarity in physical activity scores between both young and old participants.

Participants gave written-informed consent prior to undertaking any assessment, to this study, which had approval from the local university Ethics committee.

**Table 3.1.** Descriptive variables for BMI classifications in both young and old age classifications. Data are presented as Mean ± SD.

| **Young (18-49)** | Underweight (n=13) | Normal (n=13) | Overweight (n=9) | Obese (n=17) | BMI Effect | Ageing Effect |
|---|---|---|---|---|---|---|
| **Age (yrs)** | 23.0±6.7 | 23.2±7.9 | 23.6±8.0 | 30.9±10.7 | p=0.002 | p=0.001 |
| **BMI (kg/m$^2$)** | 18.8±0.9 | 21.6±1.1 | 28.1±2.4 | 35.2±4.4 | p<0.001 | p=0.625 |
| **Body Fat %** | 26.5±3.9 | 30.4±3.5 | 38.7±5.9 | 45.3±3.9 | p<0.001 | p=0.002 |
| **Fat Mass (kg)** | 13.7±2.2 | 17.2±2.7 | 28.5±6.8 | 43.2±7.3 | p<0.001 | p=0.166 |
| **ASM (kg)** | 15.8±1.8 | 16.1±2.6 | 18.7±2.7 | 21.3±3.5 | p<0.001 | p<0.001 |
| **ASM/height$^2$ (kg/m$^2$)** | 9.4±0.9 | 9.8±1.1 | 11.5±1.4 | 12.8±1.8 | p<0.001 | p<0.001 |

| **Old (50-80)** | Underweight (n=4) | Normal (n=15) | Overweight (n=18) | Obese (n=11) | BMI Effect | Ageing Effect |
|---|---|---|---|---|---|---|
| **Age (yrs)** | 63.8±5.7 | 63.5±7.7 | 68.2±4.8 | 62.5±9.0 | p=0.183 | p=0.001 |
| **BMI (kg/m$^2$)** | 19.1±0.8 | 22.2±1.0 | 27.3 ±1.2 | 34.1±5.7 | p<0.001 | p=0.625 |
| **Body Fat %** | 26.5±2.1 | 36.0±3.6 | 42.9±3.3 | 46.1±5.0 | p<0.001 | p=0.002 |
| **Fat Mass (kg)** | 12.5±2.0 | 19.9±2.9 | 29.8±3.4 | 40.9±11.3 | p<0.001 | p=0.166 |
| **ASM (kg)** | 14.4±1.2 | 13.9±1.2 | 15.2±1.6 | 18.5±3.7 | p=0.001 | p<0.001 |
| **ASM/height$^2$ (kg/m$^2$)** | 9.0±0.7 | 8.7±0.6 | 9.4±0.9 | 11.4±1.8 | p=0.001 | p<0.001 |

(Appendicular skeletal muscle mass = ASM)

## Body Composition Measure

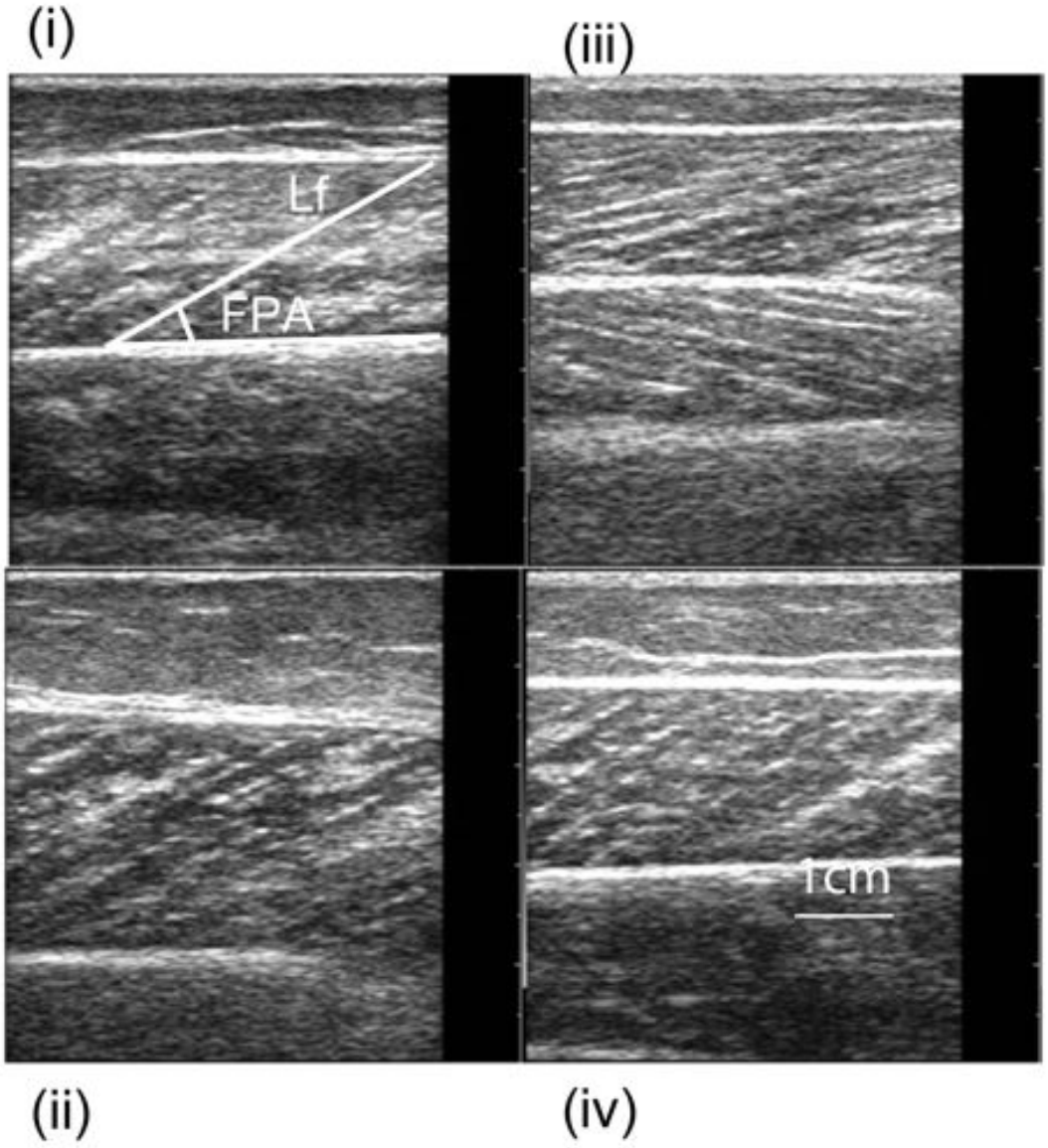
A Dual Energy X-ray Absorptiometry (DEXA) scanner (Hologic Discovery: Vertec Scientific Ltd, UK) was used to ascertain 12 hours fasted whole body composition. Participants lay in a supine position, avoiding any contact between the trunk and the appendicular mass during a 7 min scanning procedure (whole body procedure, EF 8.4 μSv). Appendicular skeletal muscle mass (ASM) was estimated from the DEXA as the total muscle mass of both the upper and lower limbs. The appendicular skeletal muscle mass index was then calculated using the following calculation - ASM/height$^2$ (kg/m$^2$).

**Muscle Architecture**

Muscle architecture of the gastrocnemius medialis (GM) was measured using B-mode ultrasonography (AU5 Harmonic, Esaote Biomedica, Genoa, Italy) at both rest and during a graded maximal MVC over 6 seconds. Participants were seated in an isokinetic dynamometer (Cybex Norm, Cybex International, New York, NY) with their hip at 85° angle, and dominant leg extended and with their foot secured to the footplate of the dynamometer. Participants were strapped into the dynamometer using inextensible straps at the hip, distal thigh and chest to reduce extraneous movements.

Resting fascicle pennation angle (FPA) and fascicle length (Lf) were measured with the probe (7.5 MHz linear array probe, 38 mm wide) positioned at 50% of the GM muscle length, at mid muscle belly in the sagittal plane as shown in Figure 3.1. Participants were then asked to perform a ramped MVC over 6 seconds, where the change in both FPA and Lf were recorded on the capturing software (Adobe Premier pro Version 6, Adobe Systems Software, Ireland). Both resting and maximal images (the latter synchronised with torque outputs using a square wave signal generator) were extrapolated from the capturing software and analysed using ImageJ (1.45s; National Institutes of Health, Bethesda, Maryland). Three clearly visible fascicles within the capturing window were defined from the deep to the superficial aponeurosis were analysed and the mean value of Lf and FPA were recorded. FPA was defined as the angle that the fascicular path undertook from the superficial to the deep aponeuroses (datum line) of the GM muscle. Linear extrapolation was used on fascicles that extended off the edge of the screen. Extrapolation was only undertaken if 60% of the chosen fascicle was visible within the scanning window in line with previous methodology examining muscle architecture of the GM in both a young and old population (Morse et al. 2005a).

**Figure 3.1**. Representative sagittal plane sonographs of the gastrocnemius medialis at 50% of its muscle length in a (i) young normal weight female, (ii) young obese female, (iii) old normal weight female and (iv) old obese female (FPA = fascicle pennation angle; Lf = fascicle length).




**Muscle Volume**

GM muscle volume was calculated using the truncated cone method through the construction of several ACSA's taken at discrete muscle sites (25, 50, and 75% of GM length) using B-mode ultrasonography (AU5 Harmonic, Esaote Biomedica, Genoa, Italy). Participants lay in the prone position with their ankle positioned in neutral (90 degrees angle, referred here as 0 degrees). B-mode

ultrasonography was then used to ascertain the proximal insertion (0% of total length) and distal insertion (100% of total length) of the GM, where discrete muscle sites (0, 25, 50, 75% and 100% of length) were marked from the medial to lateral border of the GM. Thin strips (2mm) of micropore tape (3M, Bracknell, UK) were placed axially 3-4cm apart, transversally along the nominated muscle lengths (see Figure 3.2). The micropore tape was utilised as an echo-absorptive marker in the schematic reconstruction of ACSA's using photo editing software (Adobe Photoshop; Version 10). During recording of the ACSA the ultrasound probe (7.5 MHz linear array probe, 38 mm wide) was held perpendicular to the GM on its medial border and moved along a designated marked pathway to its lateral border to ensure the probe was kept perpendicular to the GM during the whole scanning procedure. The probe was moved steadily across the leg with a constant light pressure to avoid compression of the dermal surface (and hence the muscle) during scanning. This procedure was repeated twice at each muscle site for reliability purposes.

Using the 'shadows' cast by the micropore tape as well as anatomical markers, individual transverse frames were extracted offline from each ultrasound recording to reconstruct GM ACSAs at each of the three muscle lengths of interest (Fig. 3.3) (Reeves et al. 2004a). Following this manual reconstruction of the three ACSAs at 25, 50 and 75% of muscle length, the areas of the complete transverse ACSAs were undertaken using the analysis software ImageJ (1.45s; National Institutes of Health, Bethesda, Maryland). In order to calculate the total muscle volume, an area of 0.5cm$^2$ was used as a standard measure for 0 and 100% positions along the GM muscle length. Muscle volume was then calculated using the truncated cone method (there were 4 cones in total):

Cone Volume= (⅓ x h) x π x (R1$^2$ + R1) x (R2$^2$ + R2)

Where R1 = radius of the base ACSA; R2 = radius of the top ACSA; h = distance between segments; R = √(ACSA/π), where π = 3.142

PCSA was then subsequently calculated using the ratio between GM Lf to muscle volume (PCSA = GM muscle volume (cm$^3$)/ Lf (cm)).

**Figure 3.2**. Schematic detailing the anatomical markings at the discrete muscle lengths along the gastrocnemius medialis (GM) muscle length (25%, 50% and 75%) and placement of the micropore tape. The GM insertion distal constitutes the 100% muscle length and the GM proximal insertion, the 0% length.

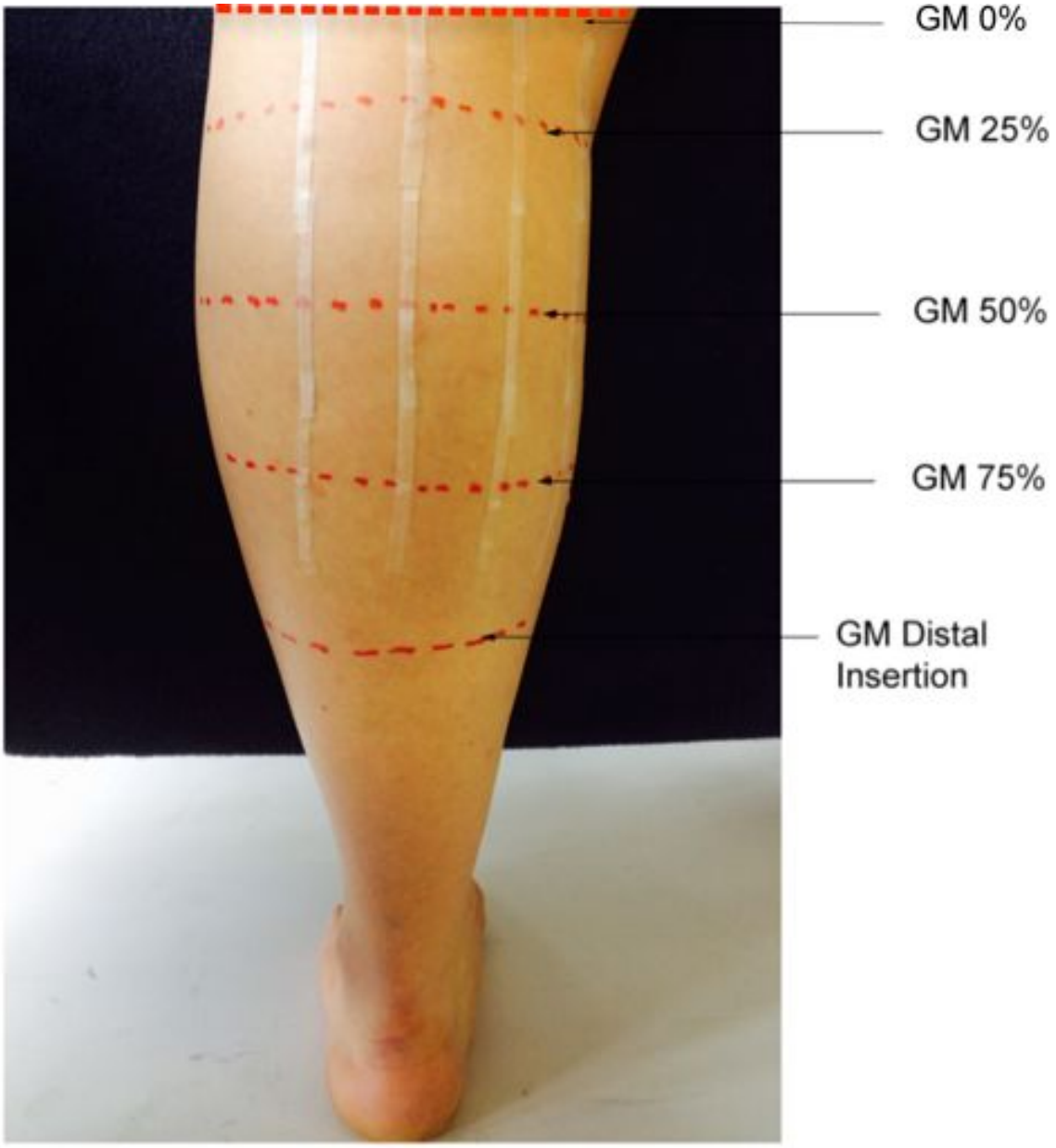
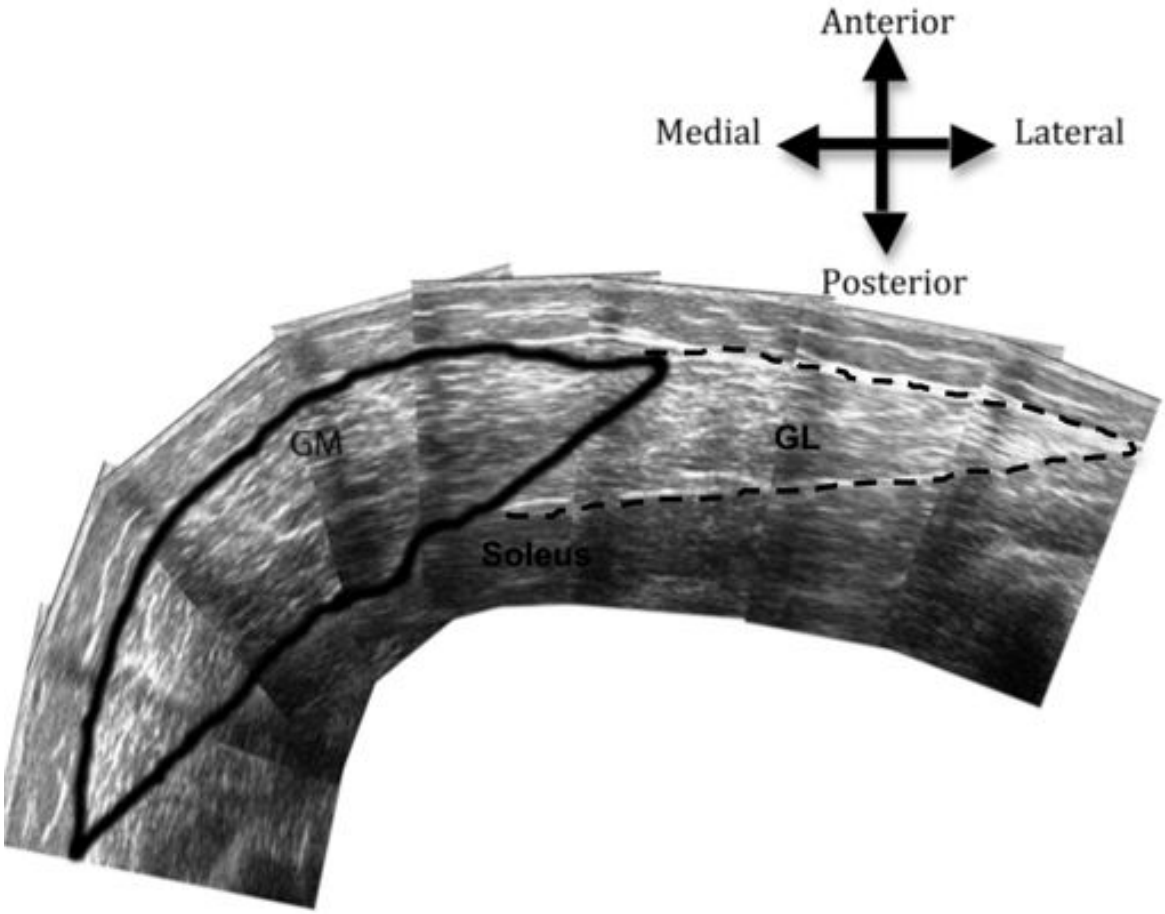


**Figure 3.3**. Reconstructed axial plane scans of the gastrocnemius medialis (GM) and gastrocnemius lateralis (GL) anatomical cross sectional area at 50% of muscle length using ultrasonography.




**Reliability**

The reliability in the measurement of both muscle architectural characteristics (muscle fascicle pennation angle and length) and GM ACSA was measured in 10 participants (Y = 5; O = 5; BMI range = 17.6-36.7) on two separate days (separated by at least 48hrs) by the same investigator.

The Intra Class Coefficients (absolute agreement) for all the measurements were high and significant for all of the assessment techniques (muscle fascicle pennation angle rest - 0.997, muscle fascicle pennation angle max - 0.997, muscle fascicle length rest - 0.996, muscle fascicle length max 0.993, GM ACSA 25% length - 0.998, GM ACSA 50% length - 0.999, GM ACSA 75% length - 0.998). It is

notable that the measurements of the ACSAs used in the construction of muscle volume are reliable and demonstrate strong agreement with MRI-obtained values (Reeves et al. 2004a).

### Statistical Analyses

Statistical analyses were carried out using SPSS (Version 19, SPSS Inc., Chicago Illinois). To determine parametricity, Kolmogorov-Smirnov (Y participants) or Shapiro-Wilk (O participants) (normal distribution) and Levene's tests (homogeneity of variance) were utilised. If parametric assumptions were met (FPA, Lf, Lf/muscle length, GM muscle volume and GM PCSA), a factorial 2 × 4 ANOVA (Age × BMI) was utilised with post hoc bonferroni correction for pairwise comparisons. Where parametric assumptions were breached (age, BMI, fat mass, ASM and ASM/height$^2$) Mann Whitney or Kruskal-Wallis test were utilised as appropriate. Pearson correlations described the relationships between measures of muscle architecture, against body mass, fat mass, total lean mass, body fat % and BMI. Comparison of the regression coefficients and slopes were conducted using z-transformations and the Student's *t*-statistic. It should be noted that some participants did not complete all tests due to faults during data capture, hence the data on regressions utilises fewer samples than the complete cohort of 100 participants (see Results Table 3.3). Data are reported as mean ± SD and statistical significance was accepted when $p \leq 0.05$. Study power ($\beta$) and effect size ($p\varepsilon^2$) are also reported.

## Results

### Body Composition

Table 3.1 displays descriptive values for age, BMI, body fat%, ASM and ASM/height$^2$ (m) for Y and O females categorised by BMI.

**Table 3.2.** Displays GM skeletal muscle characteristics (GM muscle architecture, GM anatomical cross sectional area, GM muscle volume and GM physiological cross sectional area) in both young (Y) and old (O) BMI classifications. Data are presented as Mean ± SD.

| | Young | | | | Old | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | U (n=13) | N (n=13) | O (n=9) | Ob (n=17) | U (n=4) | N (n=15) | O (n=18) | Ob (n=11) | Y BMI effect | O BMI effect | Ageing Effect |
| **FPA (°) Rest** | 18.8 ±2.5 | 17.6 ±2.9 | 21.3 ±2.9 | 21.6 ±2.3 | 15.5 ±1.0 | 17.9 ±2.2 | 19.9 ±2.8 | 21.4 ±2.7 | U N /Ob | U N /Ob | p=0.036 |
| **FPA (°) Max** | 28.4 ±5.6 | 28.3 ±3.9 | 31.4 ±4.4 | 35.2 ±4.6 | 24.5 ±3.5 | 26.4 ±3.2 | 29.3 ±4.6 | 32.3 ±3.6 | U N /Ob | U N /Ob | p=0.005 |
| **Lf (cm) Rest** | 5.2 ±0.6 | 5.3 ±0.4 | 5.5 ±0.8 | 5.7 ±0.7 | 5.7 ±0.4 | 5.4 ±0.7 | 5.4 ±1.0 | 5.4 ±0.7 | - | - | p=0.537 |
| **Lf (cm) Max** | 3.7 ±0.7 | 3.6 ±0.4 | 3.9 ±0.6 | 3.7 ±0.6 | 4.1 ±0.4 | 4.0 ±0.7 | 3.9 ±0.6 | 3.9±0.5 | - | - | p=0.063 |
| **GM 25% ACSA ($cm^2$)** | 8.4 ±2.3 | 8.7 ±2.1 | 13.8 ±5.0 | 14.0 ±2.8 | 11.2 ±2.0 | 9.7 ±2.0 | 10.2 ±2.1 | 9.7 ±2.5 | U N /Ob | - | p=0.020 |
| **GM 50% ACSA ($cm^2$)** | 12.1 ±1.9 | 13.1 ±2.6 | 17.1 ±4.2 | 21.3 ±4.7 | 12.4 ±1.4 | 13.7 ±2.3 | 14.8 ±3.6 | 16.9 ±4.0 | U N /Ob | - | p=0.110 |
| **GM 75% ACSA ($cm^2$)** | 8.1 ±1.8 | 8.9 ±1.9 | 11.3 ±2.1 | 14.0 ±2.9 | 10.8 ±2.4 | 8.5 ±1.8 | 8.5 ±2.3 | 10.5 ±2.4 | U N O /Ob | U /Ob | p<0.001 |
| **GM Muscle Volume ($cm^3$)** | 180.4 ±38.7 | 185.0 ±37.9 | 257.5 ±83.9 | 319.4 ±56.9 | 182.4 ±27.1 | 194.0 ±40.1 | 200.1 ±39.4 | 226.3 ±48.7 | U N /Ob | - | p=0.010 |
| **GM PCSA ($cm^2$)** | 50.0 ±11.9 | 52.1 ±12.0 | 67.8 ±17.0 | 88.5 ±18.3 | 44.5 ±8.1 | 49.3 ±11.2 | 51.3 ±10.3 | 59.3 ±13.5 | U N O /Ob | - | p<0.001 |

(U= underweight, N = normal weight, O = overweight, Ob = obese) (Fascicle pennation angle = FPA) (Fascicle length = Lf)

(Anatomical cross sectional area = ACSA) (Physiological cross sectional area = PCSA)

## Muscle Pennation Angle

Muscle FPA at rest revealed a main effect of age (p=0.036; $p\varepsilon^2$=0.047; $\beta$=0.556) and BMI (p<0.001; $p\varepsilon^2$=0.337; $\beta$=1.000), but no significant age × BMI interaction (p=0.190; $p\varepsilon^2$=0.053; $\beta$=0.413). However Y obese had 16% and 24% larger muscle FPA at rest than Y underweight (p=0.020) and Y normal weight (p<0.001) individuals, whilst O obese had 38% and 20% larger muscle FPA at rest than O underweight (p=0.001) and O normal weight (p=0.005) individuals (Table 3.2)

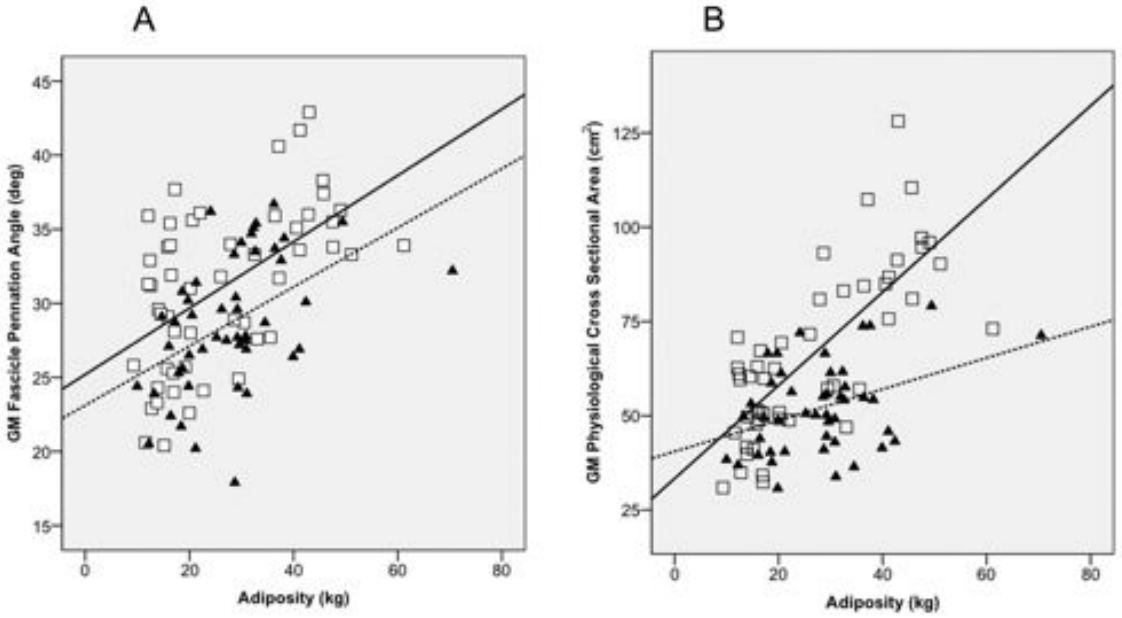
Muscle FPA during a maximum isometric contraction revealed a main effect of age (p=0.005; $p\varepsilon^2$=0.083; $\beta$=0.813) and BMI (p<0.001; $p\varepsilon^2$=0.302; $\beta$=1.000), but no significant age × BMI interaction (p=0.883; $p\varepsilon^2$=0.007; $\beta$=0.0.090). However Y obese had 25% and 25% larger muscle FPA during a maximum isometric contraction than Y underweight (p=0.001) and Y normal weight (p=0.001) individuals, whilst O obese had 32% and 22% larger muscle FPA during a maximum isometric contraction than O underweight (p=0.008) and O normal weight (p=0.003) individuals (Table 3.2).

**Muscle Fascicle Length**

Muscle Lf at rest revealed no significant effects of age (p=0.537; $p\varepsilon^2$=0.004; $\beta$=0.094), BMI (p=0.789; $p\varepsilon^2$=0.011; $\beta$=0.116) nor age × BMI interaction (p=0.227; $p\varepsilon^2$=0.041; $\beta$=0.339) (Table 3.2).

Similarly, muscle Lf during a maximum isometric contraction revealed no significant effects of age (p=0.063; $p\varepsilon^2$=0.037; $\beta$=0.461), BMI (p=0.376; $p\varepsilon^2$=0.021; $\beta$=0.185) nor age × BMI interaction (p=0.653; $p\varepsilon^2$=0.017; $\beta$=0.158) (Table 3.2).

**Figure 3.4.** Displays the impact of fat mass on gastrocnemius medialis (GM) fascicle pennation angle during maximum isometric contraction and GM physiological cross sectional area in both young (□ —— A: $r^2$ = 0.303; p<0.001; B: $r^2$ = 0.569; p<0.001) and old (▲ ------- A: $r^2$ = 0.223; p=0.001; B: $r^2$ = 0.149; p=0.009) females.



**Muscle Anatomical Cross-Sectional Area**

GM ACSA at 25% of muscle length revealed a main effect of BMI ($p<0.001$; $p\varepsilon^2=0.217$; $\beta=0.988$), an age effect ($p=0.020$; $p\varepsilon^2=0.061$; $\beta=0.650$), as well as an age × BMI interaction ($p=0.001$; $p\varepsilon^2=0.179$; $\beta=0.961$). This translated to Y obese having 68% and 61% greater GM ACSA than Y underweight ($p<0.001$) and Y normal weight ($p<0.001$) individuals, whilst O obese individuals did not have significantly greater ACSA than their underweight, normal weight and overweight counterparts ($p>0.05$) at that site (Table 3.2).

GM ACSA at 50% of muscle length revealed a main effect of BMI ($p<0.001$; $p\varepsilon^2=0.365$; $\beta=1.000$), no significant age effect ($p=0.110$; $p\varepsilon^2=0.029$; $\beta=0.359$) and no age × BMI interaction ($p=0.059$; $p\varepsilon^2=0.081$; $\beta=0.617$). This translated to Y obese having 76% and 62% greater GM ACSA than Y underweight ($p<0.001$) and Y normal weight ($p<0.001$) individuals, whilst O obese individuals did not have significantly greater ACSA than their underweight, normal weight and overweight counterparts ($p>0.05$) (Table 3.2).

GM ACSA at 75% of muscle length revealed a main effect of BMI ($p<0.001$; $p\varepsilon^2=0.371$; $\beta=1.000$), an age effect ($p<0.001$; $p\varepsilon^2=0.144$; $\beta=0.968$), yet, no age × BMI interaction ($p=0.062$; $p\varepsilon^2=0.080$; $\beta=0.609$). More specifically, Y obese had 74%, 58% and 24% greater GM ACSA than Y underweight ($p<0.001$), Y normal weight ($p<0.001$) and Y overweight ($p=0.048$) individuals, whilst O obese individuals only had 2% lower ACSA than their underweight counterparts ($p=0.046$) (Table 3.2).

**Muscle Volume**

GM muscle volume data revealed a main effect of age ($p=0.010$; $p\varepsilon^2=0.074$; $\beta=0.745$), BMI ($p<0.001$; $p\varepsilon^2=0.354$; $\beta=1.000$) and an age × BMI interaction ($p=0.003$; $p\varepsilon^2=0.145$; $\beta=0.897$). Thus, Y obese had 77% and 73% greater GM muscle volume than Y underweight ($p<0.001$) and Y normal weight ($p<0.001$) individuals, whilst O obese individuals did not have significantly greater GM muscle volume than their underweight, normal weight and overweight counterparts ($p>0.05$) (Table 3.2).

**Muscle Physiological Cross-Sectional Area**

GM PCSA revealed a main effect of age ($p<0.001$; $p\varepsilon^2=0.185$; $\beta=0.992$), BMI ($p<0.001$; $p\varepsilon^2=0.371$; $\beta=1.000$) and an age × BMI interaction ($p=0.004$; $p\varepsilon^2=0.141$; $\beta=0.882$). Specifically, Y obese had 77%, 70% and 31% larger GM PCSA than Y underweight ($p<0.001$), Y normal weight ($p<0.001$) and Y overweight ($p=0.017$) individuals, whilst O obese individuals did not have significantly larger GM PCSA than their underweight, normal weight and overweight counterparts ($p>0.05$) (Table 3.2).

**Associations between muscle architecture and body composition according to age**

Muscle FPA during a maximum isometric contraction and FM were correlated in both the Y ($p<0.001$; $r^2 = 0.303$) and O ($p=0.001$; $r^2 = 0.223$) age groups, with similar slopes in the two age groups (Figure 3.4.A). Similar correlations were observed during resting conditions between skeletal muscle FPA and FM in both Y ($p<0.001$; $r^2 = 0.223$) and O ($p=0.001$; $r^2 = 0.225$) groups, with similar slopes for the two age groups (Table 3.3).

There were strong positive associations between GM muscle volume and body mass, fat mass and BMI in both Y and O groups (Table 3.3). Ageing decreased the strength of the associations, in that both the correlation coefficients and the slopes of the regressions were less strong in the O group ($p<0.05$, Table 3.3).

There were strong positive associations between PCSA and body mass, fat mass and BMI in both Y ($p<0.001$) and O groups ($p=0.009$) (Table 3.3 & Figure 3.4.B). Ageing affected both the correlation coefficient in these associations ($p<0.05$) and the slope of the regressions ($p<0.05$, Table 3.3).

**Table 3.3.** Pearson correlations, z-transformation of r and Student's *t*-statistic between gastrocnemius medialis (GM) muscle volume and physiological cross sectional area (PCSA) and fascicle pennation angle (FPA) against a series of descriptive variables in young and old untrained females (* P<0.05, ** P<0.01, *** P<0.001) (If Z > 1.96, p<0.05; Z > 2.58, p<0.01) (Student's *t*-statistic significance if *t* falls outside ± 1.96 p<0.05)

| | Young | | | Old | | | Correlation co-efficient | Ageing Effect |
|---|---|---|---|---|---|---|---|---|
| | n | r value | slope | n | r value | slope | Z-transformation of r | Student's *t*-statistic |
| **GM Muscle Volume vs. BM** | 50 | 0.82*** | 3.15$cm^3$/kg | 45 | 0.47** | 1.19 $cm^3$/kg | 2.39* | 3.15* |
| **GM Muscle Volume vs. FM** | 50 | 0.76*** | 4.54 $cm^3$/kg | 45 | 0.40** | 1.52 $cm^3$/kg | 2.37* | 3.90* |
| **GM Muscle Volume vs. BMI** | 50 | 0.75*** | 8.23$cm^3$/kg/$m^2$ | 45 | 0.43** | 3.13$cm^3$/kg/$m^2$ | 2.07* | 3.51* |
| **GM PCSA vs. BM** | 49 | 0.81*** | 0.86$cm^2$/kg | 45 | 0.45** | 0.32$cm^2$/kg | 2.45* | 2.61* |
| **GM PCSA vs. FM** | 49 | 0.75*** | 1.24$cm^2$/kg | 45 | 0.39** | 0.41$cm^2$/kg | 2.34* | 3.77* |
| **GM PCSA vs. BMI** | 49 | 0.72*** | 2.17$cm^2$/kg/$m^2$ | 45 | 0.39** | 0.80$cm^2$/kg/$m^2$ | 2.02* | 3.26* |
| **FPA (rest) vs. BM** | 51 | 0.50*** | 0.73deg/kg | 48 | 0.49** | 0.89deg/kg | 0.03 | -4.79* |
| **FPA (rest) vs. FM** | 51 | 0.47*** | 0.11deg/kg | 48 | 0.48** | 0.13deg/kg | 0.07 | 0.50 |
| **FPA (rest) vs. BMI** | 51 | 0.53*** | 0.22deg/kg/$m^2$ | 48 | 0.52*** | 0.27deg/kg/$m^2$ | -0.02 | 0.55 |
| **FPA (max) vs. BM** | 51 | 0.60*** | 0.16deg/kg | 48 | 0.52*** | 0.15deg/kg | 0.43 | 0.26 |
| **FPA (max) vs. FM** | 51 | 0.55*** | 0.23deg/kg | 48 | 0.47** | 0.20deg/kg | 0.36 | 0.35 |
| **FPA (max) vs. BMI** | 51 | 0.57*** | 0.43deg/kg/$m^2$ | 48 | 0.50*** | 0.40deg/kg/$m^2$ | 0.43 | 0.21 |

(Body mass = BM) (Fat mass = FM) (Body mass index = BMI)

## Discussion

The data supports the hypothesis that high body mass (and/or high BMI and/or high levels of adiposity (absolute fat mass)), acts as a loading stimulus to the GM muscle, particularly in the young. Indeed, GM muscle PCSA, volume and FPA were significantly higher in the Y obese women compared to their normal weight counterparts. Interestingly, even though GM muscle FPA was found to increase, muscle Lf did not change with BMI. This effect, functionally, would translate into a potential for increased force but not increased speed of contraction with obesity.

Irrespective of BMI, there were no significant differences in muscle Lf between Y and O individuals. However as expected, Y individuals had significantly higher GM PCSA, GM muscle volume and muscle FPA compared to O. Interestingly, there were significant differences in the positive association between PCSA and BMI, and between body mass and fat mass, in Y compared with O individuals. This suggests that the loading stimulus of high body mass (and particular where this is associated with high levels of adiposity) is partially blunted in the O cohort, possibly through higher levels of circulating pro-inflammatory cytokines and/or lower anabolic growth hormones previously associated with ageing and obesity (Schrager et al. 2007).

### Muscle Architecture

To my knowledge, this is the first study to compare muscle architecture in non-obese vs. obese human adults. This study confirms previous reports (Narici et al. 2003) that muscle FPA decreases with age (Table 3.2), yet muscle Lf does not change with age or BMI classification (Table 3.2).

It was found that muscle FPA at rest and during maximum muscle contraction increases with BMI classification in both Y (rest 15%, 23% and 1%; max 25%, 25% and 13%) and O (rest 38%, 20% and 8%; max 32%, 22% and 10%) individuals (for underweight, normal, overweight people, respectively, Table 3.2). An increase in FPA allows for more sarcomeres to be arranged in parallel, which in humans suggests hypertrophy at the single fibre level (Clark et al. 2011). This in turn enables an increase in MVC torque, as long as an increase in FPA does not exceed 45° at which point the resultant force resolved at the tendon becomes negative (Alexander and Vernon 1975; Degens et al. 2009). This finding is emphasised in Figure 3.4.A, demonstrating that as fat mass increases, muscle

FPA in both Y ($r^2$=0.303; p<0.001) and O ($r^2$=0.223; p=0.001) individuals increases. Within this association there were no differences in the slope of the regression or comparison of the correlation coefficients between age categories (p>0.05) suggesting the loading effect of adiposity on muscle FPA is similar in Y and O individuals'. These increases in FPA both at rest and during maximal contraction reflect the responses seen in bodybuilders, who chronically load their musculature with weight with the aim of increasing muscle mass and have been shown to possess a greater FPA when compared to normal weight controls (Kawakami et al. 1993).

Whether the obesity-mediated beneficial increases in FPA allows more contractile material between the aponeuroses (which is likely to be indicative of fibre hypertrophy as observed in diet-induced obesity in pigs (Clark et al. 2011)), and whether this effect is the same in both Y and O obese individuals, remains to be confirmed. Alternatively, obesity could cause pseudo-hypertrophy, whereby excessive fat infiltrates the muscle, thus artificially increasing muscle thickness and altering the fascicle pennation angle. Fat infiltration has previously been reported in the skeletal musculature of the elderly (Borkan et al. 1983; Delmonico et al. 2009; Visser et al. 2005), and is linked to a lowering of the intrinsic force generating capacity of the whole muscle (Morse et al. 2005b).

There were no differences in muscle Lf between either Y and O individuals (p=0.063) or BMI sub-categories (p=0.376). As this was the first study to examine the effect of adiposity on muscle fascicle geometry, there appears to be no research to compare the effect of adiposity on Lf. Nevertheless, it is notable that research examining the ageing response on fascicle geometry, reports varying results in the gastrocnemius. For instance Kubo *et al*. (2003) reported both GM muscle FPA (r=-0.112; p>0.05) and Lf (r=-0.109; p>0.05) to not change as a result of ageing, whereas Morse *et al*. (2005a) revealed both gastrocnemius lateralis muscle FPA (-13%) and Lf (-16%) to significantly decrease with ageing. Briefly, the physiological implication of a shortened Lf is a decrease in the number of sarcomeres in series, with a potential twofold effect: (a) an alteration to the working range of the muscle, where this unit may adapt by exhibiting a change in its force-length relationship, shifting to a shorter muscle length for peak force; (b) a decrease in the muscle shortening velocity, and ultimately the muscle maximum power generation capacity. This cascade of effects would potentially cause problems for an obese or elderly population in activities such as locomotion and

tasks involving the need to apply forces and relatively high velocities (such as, in getting up from a chair to answer a doorbell ring for instance).

In the current study, the mean (across all BMI categories) GM muscle FPA during a maximum contraction decreased significantly with ageing (-8%) similar to the -16% ageing-related FPA decrease reported by Morse *et al*. (2005a), suggesting a loss of sarcomeres in parallel. A dissociation between fascicle length and pennation angle changes is not unique to the present study. For instance, a 12-month resistance-training program in the elderly, highlighted increases in muscle FPA (12% vs. 19%), yet no alterations in muscle Lf (Morse et al. 2007).

**Muscle Size**

Prior to the present study, there appeared to be no information on the effect of body composition on PCSA. This data, which employed an accurate, non-invasive measure of muscle size, revealed main effects of BMI ($p<0.001$) and ageing ($p<0.001$), as well as a BMI x age interaction ($p=0.004$) for PCSA differences. Thus, we demonstrate that adiposity places a loading stimulus similar to that attained with resistance training in Y (Erskine et al. 2010b), more so than O (Morse et al. 2007) individuals (Table 3.2). However, within the older cohort, the blunted response maybe explained through the older muscle being unable to adapt to the load placed upon the musculature. These findings support the work by Lafortuna *et al.* (2013), who reported the continuum of increasing BMI from normal weight to obese individuals to increase absolute lower limb muscle volume. However, Lafortuna *et al.* (2013) used a small sample (n=18), as well as narrower age range (32-76 years old females) in comparison to the present study.

In addition to the BMI x age interaction, the slopes of the regressions between BMI, body mass or adiposity and PCSA were steeper in Y vs. O (Table 3.3 & Figure 3.4.B), thus highlighting the lower response to the loading effect from body mass/adiposity in the older cohort. The plasticity of the younger muscle appears to structurally adapt similar to a resistance trained muscle, yet the older musculature is unable adapt to the loading. Reduced muscle mass is a known characteristic of sarcopenia in the elderly (Roubenoff 1999) and is demonstrated in this study (-20% normal BMI O vs. normal BMI Y) even though the O females did not match the sarcopenic criterion (9.6 ± 1.5 kg/m$^2$ in this group, vs. ≤5.67 kg/m$^2$ standard (Baumgartner et al. 1998)). Yet, the decreased GM PCSA was exacerbated in the obese O females (assuming a linear regression when

compared against their underweight, normal weight and overweight counterparts). A plausible rationale for the greater loss in PCSA between Y and O obese individuals may be explained through higher levels of circulating pro-inflammatory cytokines seen in both obese and sarcopenic obese individuals (Schrager et al. 2007; Hotamisligil et al. 1995). Increases in inflammatory cytokines such as interlukin-6 (IL-6) and tumour necrosis factor α (TNF-α), have been shown to negatively correlate with muscle strength and lower muscle mass in the elderly (Visser et al. 2002). High levels of these specific cytokines expressed by adipose tissue seen in obesity (Schrager et al. 2007) are reported to increase catabolic activity of skeletal muscle (Roubenoff et al. 1997). In addition to increased catabolic activity, reduced anabolic signalling of growth hormones such as insulin like growth factor-1 (IGF-I) are reported in both elderly (Bucci et al. 2013) and severely obese male and females (Williams et al. 1984). Therefore, the potential synergistic action of increased catabolism and decreased anabolism may explain 'combined ageing and obesity'-induced losses in GM muscle tissue content, which are over and above expected 'normal ageing'-related decrements.

Future research would need to confirm the co-existence of high pro-inflammatory cytokines milieu, with decreased anabolic potential, in ageing-with-obesity. Based on such endocrine investigations into pro-inflammatory cytokines such as TNF-α and IL-6, it would then be possible to substantiate the interaction of the two factors (ageing and obesity), in blunting the myogenic response associated with increased mechanical loading (in this case, through additional body fat), observed in this study.

## Conclusion

This study for the first time demonstrates that PCSA and FPA of the GM adapts to the loading stimulus of high BMI and/or adiposity in obese young and old females. Increases in GM PCSA and volume when correlated with either BMI and body or fat mass differed between the young and old obese females. The younger muscle mass was seen to adapt to the loading created by high levels of BMI and/or adiposity by increasing GM muscle volume and increasing its FPA to produce higher maximum torque. This adaptation however, does not appear to occur in older obese persons. Nonetheless, the older cohort increased their FPA to the same extent as the young women, which may explain an increase in maximum torque in the obese old relative to other BMI/adiposity classifications of

older women. These findings are suggestive of differential rate of skeleto-muscular ageing, dependent on a person's body composition. Therefore, there is a case for implementing different exercise and/or nutrition interventions according to the somatotype and age of the individual concerned.

# Chapter 4: Obesity decreases whole muscle strength in young females and exacerbates the ageing-related whole muscle level asthenia

Data as presented in: Tomlinson DJ, Erskine RM, Winwood K, Morse CI, Onambele GL (2014b) Obesity decreases both whole muscle and fascicle strength in young females but only exacerbates the ageing-related whole muscle level asthenia. Physiol Rep 2 (6). doi:2/6/e12030 [pii]10.14814/phy2.12030

## Abstract

Obesity has previously been associated with greater muscle strength. Ageing, on the other hand, reduces muscle specific force (the force per unit physiological cross-sectional area [PCSA] of muscle). However, neither the effect of obesity on skeletal muscle specific force nor the combined effects of ageing and obesity on this parameter are known. This study aimed to describe the interplay between body mass index (BMI)/adiposity, ageing, and skeletal muscle specific force. Ninety-four untrained healthy women categorised by age into young (Y; mean ± SD: 25.5 ± 9.0 years) versus old (O; 64.8 ± 7.2 years) were assessed for body composition using dual energy x-ray absorptiometry, gastrocnemius medialis (GM) muscle volume (*V*) using ultrasonography, plantar flexion net maximum voluntary contraction (nMVC) correcting for antagonist co-contraction and agonist muscle activation, and GM specific force (SF). The Y obese, while demonstrating 71% and 29% ($p<0.001$) higher *V* and nMVC compared to normal BMI individuals, were in fact 26% ($p=0.007$) weaker than these, where *V* was used to scale nMVC (i.e., nMVC/*V*). At the fascicular level, there were no significant differences in SF between obese and normal BMI individuals (-17% p=n.s.). However, ≥40% body fat was associated with 60% and 27% ($p<0.001$) higher *V* and nMVC, but 11% ($p<0.01$) lower nMVC/*V* than <40% body fat. The ageing-related rates of decline were foremost observed in *V* (-2.1 $cm^3$/year $p<0.05$) and nMVC (-1.2 Nm/year $p<0.05$) in obesity defined by BMI. This effect was also seen when segregating by >40% adiposity. Interestingly, there was no effect of obesity on the ageing-related changes in SF (p=n.s).

Unlike previous reports of greater strength in the obese compared with leaner age-matched counterparts, we in fact demonstrate that the young sedentary obese, are substantially weaker, where the volume of skeletal muscle is used to scale the maximal torque output, yet not when forces are quantified at the fascicular level. The lack of impact of obesity on rate of ageing of intrinsic strength and specific force however is complex and warrants further investigations.

## Introduction

It is generally accepted that obese individuals, regardless of age, have lower maximal strength when the latter is expressed relative to body mass (Abdelmoula et al. 2012; Blimkie et al. 1990; Hulens et al. 2001; Lafortuna et al. 2005; Maffiuletti et al. 2008; Maffiuletti et al. 2007; Rolland et al. 2004). However, it is unclear whether this weakness exists at the fascicle level, or is simply a reflection of pseudo-hypertrophy whereby the relatively high amount of muscle mass in an obese person is superseded by the higher elevation in adiposity. Previous studies have shown that the maximum strength capability relative to muscle size/mass of an obese individual is not significantly different to that of normal weight individuals (Blimkie et al. 1990; Lafortuna et al. 2005; Maffiuletti et al. 2008; Maffiuletti et al. 2007). This, however, could be a potential type II error due to the use of total fat-free mass (Lafortuna et al. 2005; Maffiuletti et al. 2008; Maffiuletti et al. 2007) and anatomical cross-sectional area (ACSA) (Blimkie et al. 1990) as indices of muscle size. This can be explained by the aforementioned measures not accounting for the architectural characteristic of the skeletal muscle responsible for the joint action, thus potentially misconstruing the true amount of muscle that contributes to torque production (Alexander and Vernon 1975). However, contradictions also exist in the literature with previous authors (Hulens et al. 2001) reporting that obese adult individuals have 6–7% lower torque relative to total fat-free mass and hypothesising that this effect may be due to an assumed reduced agonist muscle activation. Indeed lower agonist muscle activation has been recorded in both obese adolescent (Blimkie et al. 1990) and obese adult populations (Chapter 2). Contrary to the above, other authors (Abdelmoula et al. 2012) reported obese adolescents to have 22% higher knee extension torque normalised to thigh lean muscle mass (data obtained from dual energy x-ray absorptiometry [DEXA]). In fact, the only study to have utilised a more accurate and valid method of measuring the muscle mass involved in isometric torque production was that of Hilton et al. (2008) who assessed the muscle volume of the triceps surae in obese individuals. These authors (Hilton et al. 2008) demonstrated that obese individuals produced lower torque relative to muscle volume. However, the participants recruited had diabetes mellitus and peripheral neuropathy, both of which have previously been shown to independently cause motor weakness (Andersen et al. 1996).

To my knowledge, to date, no obesity/strength interaction study has accounted for the pennate architecture of the antigravity musculature of the lower

limbs in the estimation of muscle size. An accurate quantification of muscle size is its physiological cross-sectional area (PCSA; volume ÷ fascicle length), as this represents the number of sarcomeres in parallel, and exhibits a linear association with a muscle's maximum force capability (Fukunaga et al. 2001). Skeletal muscle specific force (force per unit PCSA) depicts an accurate representation of a muscle's maximum strength capacity, as it corrects for both the physiological and biomechanical determinants of maximal muscle strength (Erskine et al. 2009; Maganaris et al. 2001; Reeves et al. 2004b).

Ageing has been shown to reduce skeletal muscle specific force (Morse et al. 2005b). This reduction has been hypothesised as being due to a decline in the muscle activation capacity of elderly individuals, partly owing to lower habitual physical activity levels (Morse et al. 2004). In addition, preferential muscle fibre atrophy (Lexell and Taylor 1991) and a decrease in number of type II fibre (Lexell 1995) accompanied with an increase in intramuscular fatty infiltration (Rice et al. 1989), are likely to play a role in the ageing-related lowering of muscle specific force. Yet, it appears that no investigation has systematically examined whether, after accounting for neural and architectural factors underlying muscle force production, the effects of ageing would be exacerbated in the presence of obesity, thereby leading to a greater drop in maximal force generation capability of the old obese compared to their normal weight, age-matched counterparts. The increased prevalence of obesity (James 2008), which accompanies the rising level in life expectancy, renders the determination of therapeutic interventions to reverse the deleterious effects of the two “conditions” extremely timely.

Therefore, the aim of the present study was to investigate the degree of impact of obesity on skeletal muscle intrinsic force at both whole muscle and fascicular levels, in a young inactive population. The second aim was to determine whether the effects of ageing and adiposity are in fact additive on skeletal muscle specific force at both whole body and fascicle levels. We hypothesised that (1) skeletal muscle specific force in both obese Y and O would be lower when compared to lean, normal weight and overweight individuals, where muscle strength is quantified at both gross and fascicular levels; (2) the deleterious impact of high adiposity on skeletal muscle specific force (at both gross and fascicular levels), would be worse in the older individuals and their younger counterparts; and (3) the rate of ageing (decrease in muscle contractile capacity) would be faster in the presence of obesity.

## Methods

### Ethical approval:

Participants gave written informed consent prior to undertaking any assessment. All the procedures in this study had approval from the Manchester Metropolitan University Ethics committee and conformed to the standards set by the latest revision (2012-2013) of the Declaration of Helsinki.

### Participants:

The current study was on a single gender basis in order to minimize the potential confounding effect of male versus female differential rate of ageing (Lindle et al. 1997), and/or sensitivity to adiposity (Lafortuna et al. 2005; Lafortuna et al. 2013). Thus, 94 untrained females volunteered to take part in this study (Table 4.1 & 4.2) and were categorised by age into either young (Y; 18–49 years) or old (O; 50–80 years). As 91/94 participants were white Caucasians, no sub grouping by ethnicity was carried out. Participants were then subcategorised into four body mass index classifications (BMI – body mass [kg]/stature$^2$ [m]) into underweight (BMI <20), normal (BMI 20–24.9), overweight (BMI 25–29.9), and obese (BMI >30). Participants were also categorised as ordinary adipose (<40%) versus high adipose (≥40%) by body fat percentage following recommendations from previous studies (Rolland et al. 2009; Baumgartner et al. 2004). The exclusion criteria were any health issues highlighted in the self-reported questionnaire such as lower limb muscles/joints injuries/pathology, affecting mobility or ability to exert maximal torque. Physical activity status was screened by questionnaire and participants were excluded if they self-reported as habitually undertaking structured exercise for more than 3 h per week.

**Table 4.1.** Descriptive variables for BMI classifications in both the young (upper section) and old (lower section) age classifications (Data are presented as Mean ± SD). Rate of ageing per BMI classification is also shown (Data are presented as regression slope and p value for the degree of association).

| | | Underweight | Normal | Overweight | Obese | BMI effect | Ageing effect |
|---|---|---|---|---|---|---|---|
| **n** | **Young** | 13 | 12 | 8 | 16 | | |
| | **Old** | 4 | 14 | 16 | 11 | | |
| **BMI (Kg/m$^2$)** | **Young** | 18.8±0.9 | 21.6±1.1 | 27.8±0.7 | 35.2±4.5 | p<0.001 | |
| | **Old** | 19.1±0.8 | 22.2±1.0 | 27.4±1.2 | 34.1±5.7 | p<0.001 | p= n.s. |
| **Body fat (%)** | **Young** | 26.5±3.9 | 30.1±3.5 | 37.7±5.8 | 45.5±4.1 | p<0.001 | |
| | **Old** | 26.5±2.1 | 35.6±3.4 | 43.3±2.8 | 46.1±5.0 | p<0.001 | p=0.002 |
| **Ageing slope Intrinsic strength (Ncm/V/year)** | | 0.028 (p=n.s) | -0.347 (p=n.s) | -0.261 (p<0.01) | 0.085 (p=n.s) | | |
| **Ageing slope Specific force (N/cm$^2$/year)** | | -0.006 (p=n.s) | -0.091 (p=n.s) | 0.032 (p=n.s) | 0.056 (p=n.s) | | |

(V = Gastrocnemius medialis muscle volume)

**Table 4.2.** Descriptive variables for obesity classification by body fat percentage in both the young (upper section) and old (lower section) age classifications (Data are presented as Mean ± SD). Rate of ageing per adiposity classification is also shown (Data are presented as regression slope and p value for the degree of association).

| | | Ordinary-Adipose (n=32) | High-Adipose (n=17) | Obesity effect | Ageing effect |
|---|---|---|---|---|---|
| **n** | **Young** | 32 | 17 | | |
| | **Old** | 19 | 26 | | |
| **BMI (kg/m$^2$)** | **Young** | 21.9±3.9 | 34.5±5.0 | p<0.001 | |
| | **Old** | 22.6±3.5 | 29.6±5.3 | p<0.001 | p=0.463 |
| **Body fat (%)** | **Young** | 29.9±5.1 | 45.9±3.5 | p<0.001 | |
| | **Old** | 33.6±4.7 | 44.8±3.7 | p<0.001 | p=0.006 |
| **Ageing slope Intrinsic strength (Ncm/V/year)** | | -0.278 (p<0.01) | -0.018 (p=n.s) | | |
| **Ageing slope Specific force (N/cm$^2$/year)** | | -0.097 (p=0.030) | 0.038 (p=n.s) | | |

(V = Gastrocnemius medialis muscle volume)

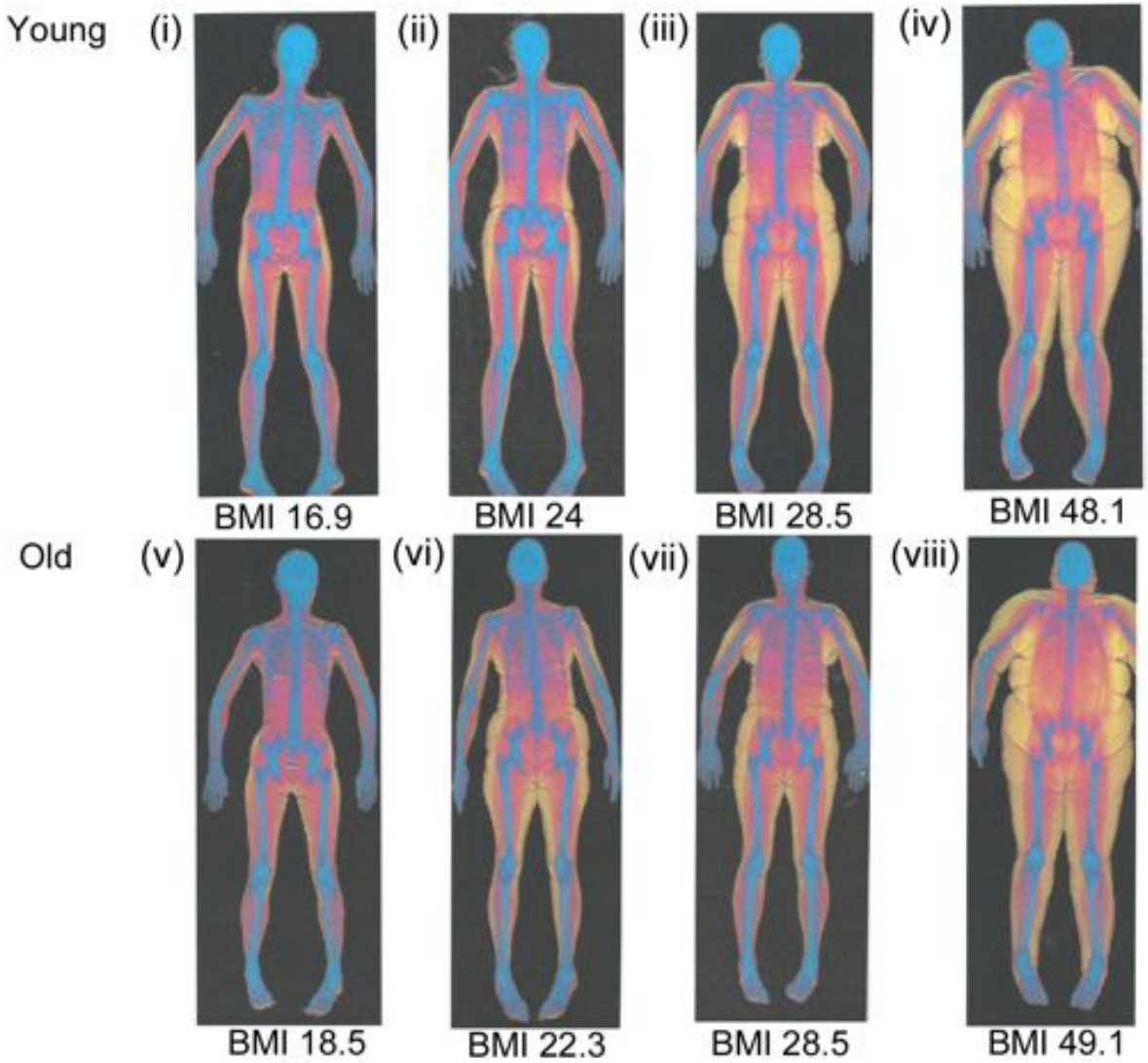
**Protocol order:**

Participants attended the laboratory for testing on two occasions. During the first visit, anthropometric measurements (DEXA, Stature, Mass) were collected, and familiarisation with the MVC protocols took place. During the second visit participants gastrocnemius medialis muscle volume and architecture data were collected alongside the main MVC protocol.

**Body Composition Measure:**

Body composition (body fat percentage, lean muscle, and bone) was ascertained using a DEXA scanner (Hologic Discovery; Vertec Scientific Ltd, Reading, U.K.) following a period of overnight fasting for 12 h. Participants lay in the supine position, avoiding any contact between the trunk and the appendicular mass during a 7-min scanning procedure (whole body procedure, effective dose 8 $\mu$Sv). Scan results were both graphical (Figure 4.1) and numerical, giving a number of descriptions of which %body fat was key for the aims in this study.

**Figure 4.1**. Representative DEXA scans of a (i) young underweight female, (ii) young normal weight female, (iii) young overweight female, (iv) young obese female, (v) old underweight female, (vi) old normal weight female, (vii) old overweight female and (vii) old obese female. Colour key: Blue for bone; Red for Lean tissue, Yellow for adipose tissue.



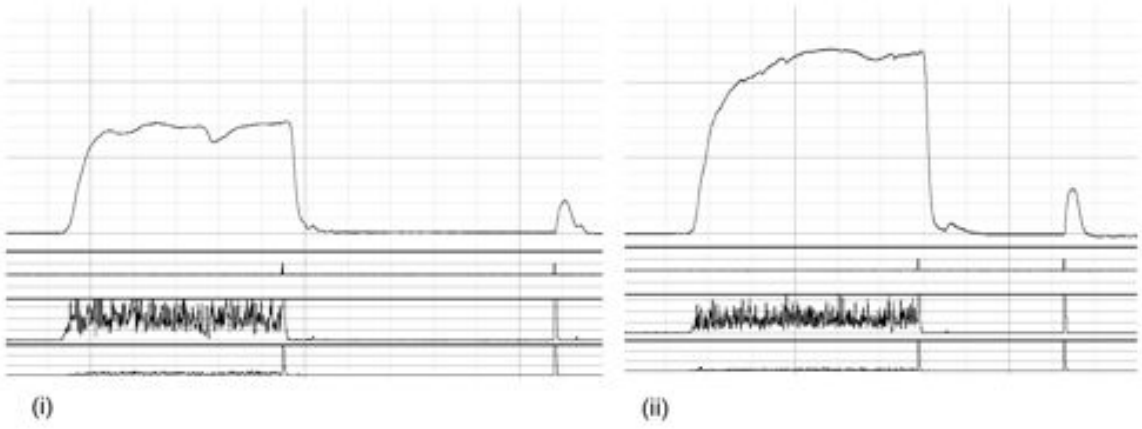

**Muscle Strength Measurement:**

Maximum voluntary contraction (MVC) torque during both ankle plantar flexion (PF) and dorsi flexion (DF) was measured in the dominant limb using an isokinetic dynamometer (Cybex Norm; Cybex International, New York, NY). Participants were seated (hip at 85° angle, dominant leg extended with the foot secured to the footplate of the dynamometer), and strapped using inextensible straps (at the hip, distal thigh, and chest) to reduce extraneous movements. Prior

to MVCs, participants were re-familiarised with the protocol undertaken during the test. Following this familiarisation protocol, participants conducted a series of five submaximal isometric contractions with their ankle positioned at 0° (anatomically neutral), starting at self-perceived 50% maximal exertion, increasing in intensity to ensure the participant was warmed up prior to maximal exertion.

During the main MVC protocol, participants were asked to conduct two (up to a maximum of four, see below) rapid isometric PF and DF MVCs with their ankle positioned at 0°, each lasting 3–4 sec. The highest (of two) recorded PF and DF MVC by the participant was utilised as their true MVC. However, MVCs were repeated if there was >10% difference between MVCs to ensure true MVC was obtained. The PF MVC was then corrected for agonist muscle activation using the interpolated twitch technique (Morse et al. 2004; Pearson and Onambele 2006) (Figure 4.2) and antagonist co-contraction of the tibialis anterior (TA) using surface electromyography (EMG). Antagonist muscle co-contraction was calculated through utilising the EMG signal (computed as root mean square) of the TA recorded 500 ms on either side of instantaneous peak torque during a maximal PF and divided by the EMG recorded during DF. The raw EMG signal measured during contractions was recorded at 2000 Hz, with band pass filter set at 10–500 Hz, and notch at 50 Hz. This calculation method assumes that the DF EMG/Torque relationship is linear (Maganaris et al. 1998). MVCs corrected for both agonist muscle activation and antagonist co-contraction (i.e., neural factors) in the manuscript are classified as net MVC (nMVC).

**Figure 4.2**. Representative Torque outputs taken during the interpolated twitch technique of a (i) young ordinary adipose female and (ii) young high adipose female.



**Muscle Volume & intrinsic strength:**

Participants lay in the prone position with their ankle positioned at 0°. B-mode ultrasonography was then used to ascertain the origin and insertion of the GM, where discrete muscle sites (25%, 50%, and 75% of length) were marked from the medial to lateral border of the GM. Thin strips (2 mm) of micropore tape (3M, Bracknell, Berkshire, U.K.) were placed axially 3–4 cm apart transversally along the nominated muscle lengths. The micropore tape was utilized as echo-absorptive marker in the formation of ACSAs from the corresponding muscle lengths when using the photo-editing software (Adobe Photoshop Elements; Version 10, Adobe Systems Incorporated, San Jose, CA). During recording of the ACSAs, the ultrasound probe (7.5 MHz linear array probe, 38-mm wide) was held perpendicular to the GM on its medial border and moved along a designated marked pathway to its lateral border. The probe was moved steadily across the leg with constant light pressure to avoid compression of the muscle during scanning. This procedure was repeated twice at each discrete muscle site for reliability purposes. The construction of the ACSAs were undertaken using Adobe Photoshop (Version 10), where still transverse images at each individual muscle length were reconstructed using the micropore tape as anatomical markers in combination with anatomical landmarks along the GM muscle length. Following construction of the three individual ACSAs, the areas of the complete transverse ACSAs were undertaken using the analysis software ImageJ (1.45s; National Institutes of Health, Bethesda, MD). Reliability in the measure of the three ACSAs was assessed in 10 participants (Y = 5; O = 5; BMI range = 17.6–36.7) on two separate days (separated by at least 48 h) by the same investigator. The intraclass coefficients were as follows: GM ACSA 25% length – 0.998, GM ACSA 50% length – 0.999, GM ACSA 75% length – 0.998. These measures are not only reliable but also externally valid as they demonstrate strong agreement with MRI-obtained values (Reeves et al. 2004a). Muscle volume was then calculated using the truncated cone method:

$$\text{Cone Volume} = (\tfrac{1}{3} \times h) \times \pi \times (R1^2 + R1) \times (R2^2 + R2)$$

Where R1 = radius of the base; R2 = radius of the top; h = distance between segments; $R = \sqrt{(ACSA/\pi)}$, where $\pi = 3.142$

Skeletal muscle intrinsic strength was quantified as nMVC relative to muscle volume.

**Calculation of GM Specific Force**

This involved several steps including the assessment of tendon force (itself requiring measurement of tendon moment arm) and muscle physiological cross-sectional area (itself requiring the assessment of muscle architecture).

Tendon excursion using B-mode ultrasonography was used to calculate the Achilles tendon moment arm length (Maganaris et al. 2000; Ito et al. 2000). Participants were seated in the isokinetic chair (Cybex Norm; Cybex International) following the experimental set up undertaken during MVCs. Prior to commencement of the protocol, the insertion of the GM muscle to the Achilles tendon was anatomically marked on the limb using micropore tape as an echo-absorptive reflective marker during recording. The ultrasound probe was then positioned across the muscle–tendon junction (MTJ) of the GM as denoted by the micropore tape. During the recording the ankle of the participant was passively rotated between 10° and −5° PF at a constant velocity of 1°/sec. The passive movement was recorded for at least three PF and DF rotations and was synchronised with torque outputs using a square wave signal generator to distinguish joint position on the capturing software.

The displacement of the MTJ of the GM between 10° PF and −5° PF was calculated using the micropore tape as a distance marker using analysis software (ImageJ 1.45s; National Institutes of Health). The Achilles moment arm length at 0° was then calculated using the displacement of the MTJ divided by the displacement in the ankle angle during a complete rotation.

$MA_{0^\circ}$ = displacement MTJ ÷ change in ankle angle

Achilles tendon force (*F*) at 0° was calculated using the PF MVC corrected for both agonist muscle activation and antagonist co-contraction, then dividing this value by the Achilles tendon moment arm length. The contribution of the GM muscle to PF MVC was calculated, assuming this muscle to contribute 25% of the total ankle plantar flexion MVC (Fukunaga et al. 1992).

$F_{GM}$ = F x 0.25, where F = nMVC ÷ $MA_{0°}$

Muscle architecture of the gastrocnemius medialis (GM) was measured using B-mode ultrasonography at both rest and during a graded isometric MVC over 6 sec. Participants were seated in an isokinetic dynamometer (Cybex Norm; Cybex International) as detailed above. The probe of a B-mode ultrasound scanner (AU5 Harmonic; Esaote Biomedica, Genoa, Italy) was positioned on the surface of the skin, at 50% of the GM muscle length, along the mid-sagittal line. Participants were then asked to perform a ramped MVC over 6 sec, where the change in both fascicle pennation angle (FPA) and fascicle length (Lf) were recorded. Images of both resting and maximal architecture were synchronised with torque outputs using a square wave signal generator, extrapolated from the capturing software (Adobe Premier pro Version 6; Adobe Systems Software, San Jose, CA) and later analyzed using ImageJ (1.45s; National Institutes of Health). Analysis of three fascicles defined from the deep to the superficial aponeurosis of the GM was then recorded and the mean value of both the FPA and Lf of the three fascicles were then recorded. If needed (in cases where the fascicles extended beyond the width of the probe), linear extrapolation of fascicles was carried out. The reliability of the measure of both FPA and Lf at rest and MVC was obtained from 10 participants (Y = 5; O = 5; BMI range = 17.6–36.7). The intraclass correlation coefficients for both the architectural measurements were high (muscle fascicle pennation angle rest – 0.997, muscle fascicle pennation angle max – 0.997, muscle fascicle length rest – 0.996, muscle fascicle length max – 0.993).

Following the calculation of GM muscle volume and architecture, PCSA was then calculated (PCSA = Muscle volume ÷ Lf).

Fascicle Force- Following the computation of the GM muscle force ($F_{GM}$), fascicle force was calculated as

GM $F_{fasc}$ = $F_{GM}$ ÷ Cos FPA

Gastrocnemius medialis muscle specific force was calculated by dividing the GM fascicle force by GM PCSA (Alexander and Vernon 1975; Fukunaga et al. 1996; Reeves et al. 2004b).

GM SF = GM $F_{fasc}$ ÷ PCSA

### Statistical Analyses

Statistical analyses were carried out using SPSS (Version 19, SPSS Inc., Chicago IL). Stem-and-Leaf plots were used to identify any outliers, and these were removed prior to further analyses. To determine parametricity, Shapiro–Wilk (normal distribution) and Levene's tests (homogeneity of variance) were used. If parametric assumptions were met, a one-way analysis of variance (ANOVA; BMI classifications) with post hoc Bonferroni correction for pairwise comparisons, or independent sample *t*-tests (%body fat classifications) were used on muscle volume, nMVC, GM intrinsic strength, and GM specific force (GM SF). Where parametric assumptions were breached, Kruskal–Wallis (BMI classifications) or Mann–Whitney (%body fat classifications) were used. Linear regressions and Pearson's moment correlations, described the relationships and the degree of association, between age and parameters of interest (including muscle volume, nMVC). Comparisons of the regression coefficients and slopes were conducted using *z*-transformations and the Student's *t*-statistic. Data are reported as mean ± SD and statistical significance was accepted when $p \leq 0.05$.

## Results

### Body Composition

Table 4.1 and 4.2 display descriptive study population characteristics of BMI and body fat% for Y and O females categorized by both BMI and %body fat.

### Skeletal Muscle Characteristics

*BMI and muscle contractile characteristics*

Figure 4.3 demonstrates the effect BMI classification has upon muscle volume, nMVC torque, intrinsic strength, and specific force in young participants. Muscle volume revealed a main effect of BMI classification ($p<0.001$). Pairwise comparisons revealed the obese females to have 75% ($p<0.001$), 71% ($p<0.001$), and 36% ($p<0.010$) greater muscle volume than their underweight, normal weight

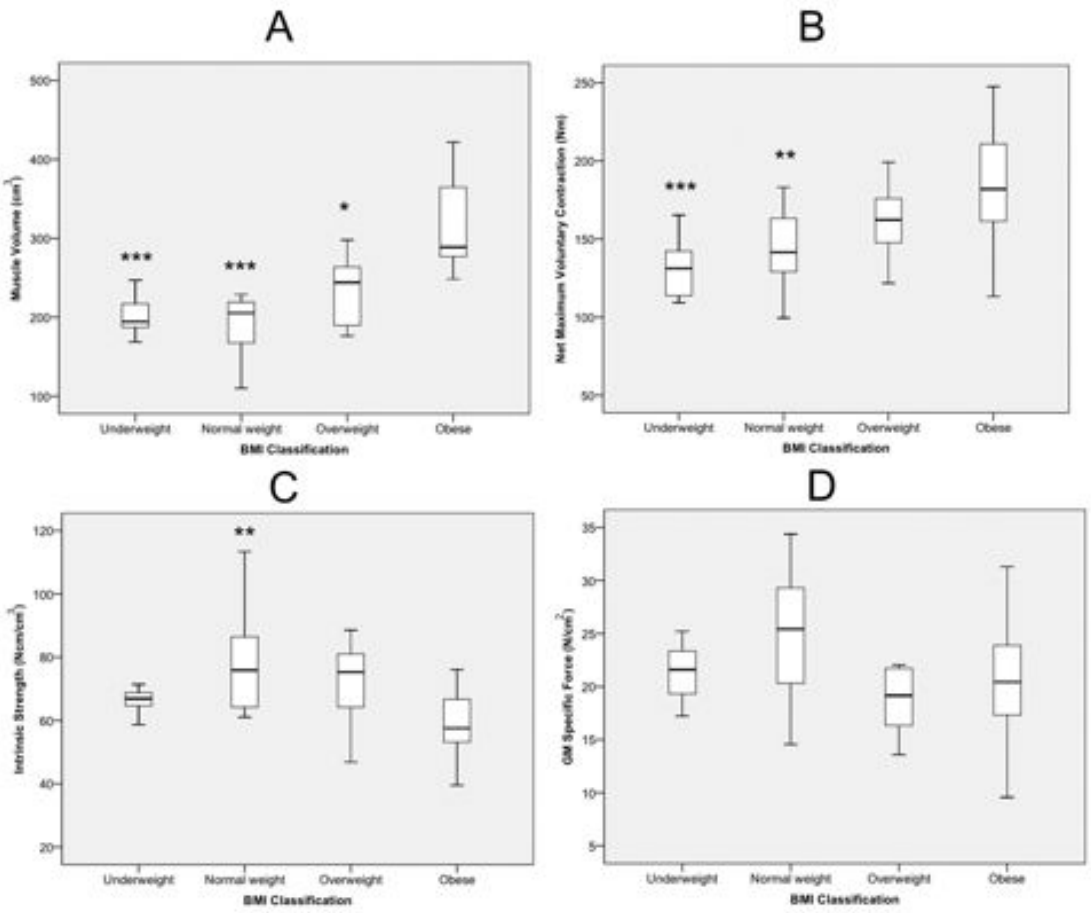
and overweight counterparts, respectively.

Net MVC torque revealed a main effect of BMI classification ($p<0.001$). Pairwise comparisons revealed the obese females to have 39% ($p<0.001$) and 29% ($p=0.005$) greater nMVC than their underweight and normal weight counterparts, respectively.

The opposite ranking order was found for indices of strength normalised for muscle content. Indeed intrinsic strength revealed a main effect of BMI classification ($p=0.005$). Pairwise comparisons revealed the obese females to have 26% ($p=0.007$) lower intrinsic strength than their normal weight counterparts. However, there was no difference in intrinsic strength between the young obese and either underweight, or overweight individuals (Figure 4.3.C).

Skeletal muscle specific force (GM SF) revealed no significant effects of BMI classification (p=n.s) (Figure 4.3.D).

**Figure 4.3.** Displays the impact of BMI classification on (A) muscle volume (minus 1 overweight and 2 obese outliers), (B) net maximum voluntary contraction (0 outliers), (C) intrinsic strength (minus 3 underweight and 2 normal weight outliers) and (D) specific force (minus 3 underweight, 1 normal weight and 1 overweight outliers) in young females (* p<0.05; * p<0.01; *** p<0.001).



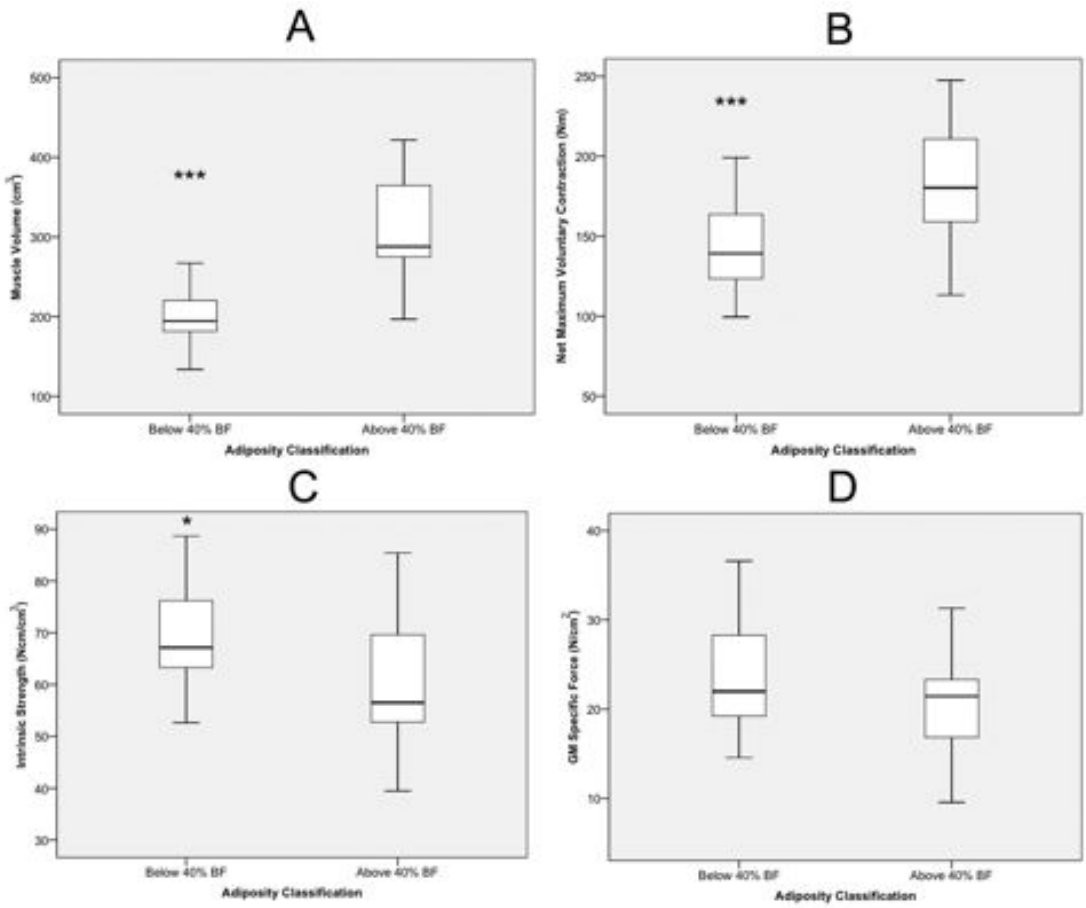

*Adiposity and muscle contractile characteristics*

Figure 4.4 demonstrates the effect that obesity classification by adiposity (i.e., %body fat) has upon muscle volume, nMVC torque, intrinsic strength, and specific force in young participants.

Highly adipose females were found to have 60% (p<0.001) greater muscle volume, and 27% (p<0.001) greater nMVC than “ordinary” adipose females.
Interestingly, however, as seen in BMI classifications, this seeming advantage of high adiposity was reversed when it was shown that the high-adipose females had in fact 11% (p=0.025) lower intrinsic strength, than their age-matched ordinary adipose-matched females than the ordinary classified females.

However, similarly to BMI classification, GM SF revealed no significant effects of adiposity classification (p=n.s).

**Figure 4.4.** Displays the impact of adiposity on (A) muscle volume (minus 1 overweight and 1 obese outliers), (B) net maximum voluntary contraction (minus 1 overweight outlier), (C) intrinsic strength (minus 3 underweight and 2 normal weight outliers) and (D) specific force (minus 1 normal weight outlier) in young females (* p<0.05; * p<0.01; *** p<0.001).




*Degree of association between age and muscle size and/or strength by obesity status*

There was a stepwise increment in the steepness of the ageing versus muscle content loss relationship, with increasing BMI. Thus, ageing-related muscle loss from the second to the seventh decade were -2.1, -0.5, 0.2, and 0.3 $cm^3$/year in the obese, overweight, normal weight, and underweight BMI categories, respectively. These differences in slopes were significant between obese and normal weight (Student's *t*-statistic 3.88; p<0.05), obese and underweight

(Student's *t*-statistic 3.64; $p<0.05$), and obese and overweight (Student's *t* statistic 2.59; $p<0.05$) females (Figure 4.5.A).

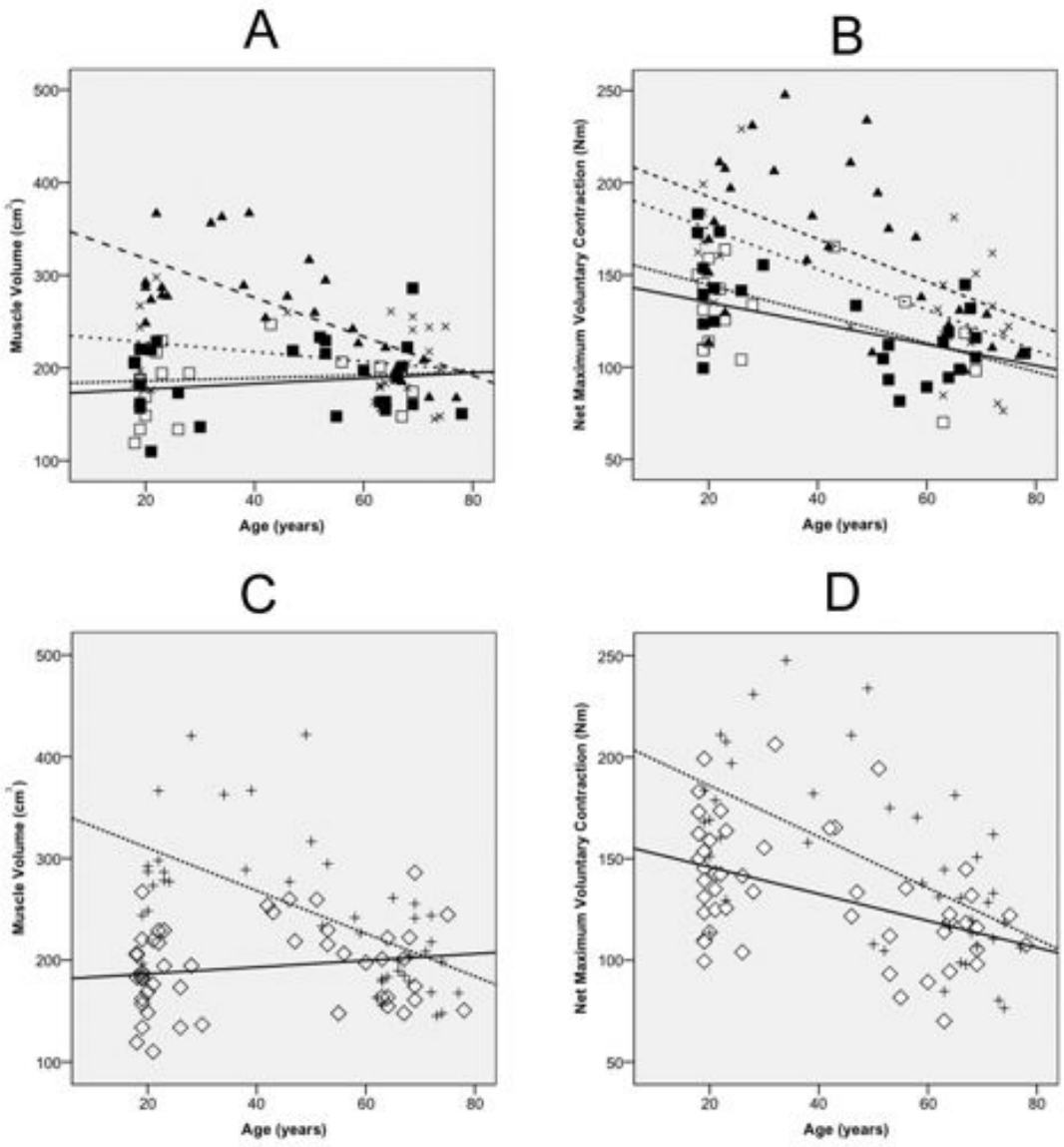
Similarly, there was a stepwise increment in the steepness of the ageing versus muscle content loss, with high adiposity. Thus, ageing-related changes from the second to the seventh decade were -2.1 versus 0.3 $cm^3$/year in high versus low %body fat categories. These differences in slopes were statistically significant (Student's *t*-statistic 4.82; $p<0.05$; Figure 4.5.C). Based on these slopes, it was evident that for muscle volume ageing was associated with faster than normal deleterious changes, regardless of whether classification was by BMI or by fat.

There was a stepwise increment in the steepness of the ageing versus nMVC loss relationship, with increasing BMI. Thus, ageing-related changes from the second to the seventh decade were -1.2, -1.1, -0.8, and -0.6 Nm/year in the obese, overweight, normal weight, and underweight BMI categories, respectively. These differences in slopes were not significantly different between BMI classifications (Figure 4.5.B).

Similarly, there was a stepwise increment on the steepness of the ageing versus nMVC loss relationship, with high adiposity. Thus, ageing-related changes from the second to the seventh decade were -1.3 versus -0.7 Nm/year in high versus low %body fat categories. However, similar to the BMI classification, this difference in the slope was not statistically significant (Figure 4.5.D).

As expected, there was a significant decrease in intrinsic strength with ageing (-0.17 Ncm/$cm^3$/year, $p=0.027$). Grouping by BMI, only highlighted a significant change with ageing in the overweight (see Table 4.1). Grouping by adiposity highlighted a significant decrease in the low-adipose group but no change in the high-adipose group (see Table 4.2).

**Figure 4.5.** Displays linear regressions (and slope comparisons) on the impact of age on both BMI and adiposity classification on muscle volume (A, C) and net maximum voluntary contraction (B, D) in sedentary females. (A) Underweight (□ —— ——) versus Obese (▲ — —) *P* < 0.05. Normal weight (■ - - - - -) versus Obese; p< 0.05. Overweight (× – – –) versus Obese p<0.05**.** (B) Underweight (□) versus Obese (▲) p>0.05; Normal weight (■) versus Obese; p>0.05; Overweight (×) versus Obese p>0.05. (C) Ordinary adipose ◊ ——; high adipose + - - - - - p<0.05; (D) Ordinary adipose versus high adipose; p>0.05.




Unexpectedly, there was no change in specific force with ageing (p>0.05). Also, grouping by BMI or adiposity highlighted no subgroup effect (see Tables 4.1 and 4.2).

## Discussion

This study is the first to systematically quantify the impact of varying levels of BMI and adiposity on skeletal muscle intrinsic (i.e., whole muscle level) and specific (i.e., fascicle level) force. When body composition status was classified by BMI, the obese cohort exhibited greater muscle volume and nMVC. Interestingly, however, the obese (compared to normal weight and underweight individuals) in fact had lower nMVC normalised to muscle volume. This significant trend was repeated when obesity was classified using adiposity, as the high-adipose female cohort (≥40% body fat) demonstrated both higher muscle volume and nMVC, yet lower nMVC normalised to muscle volume. These findings partly supported our first hypothesis.

The rate of ageing in terms of both muscle volume and nMVC was found to be worst in the obese cohort whether classified by BMI or adiposity, again supporting one of our hypotheses. However, contrary to our final hypothesis, a decrease in intrinsic strength was only apparent in the pooled population (regardless of obesity status). Where the data were grouped by BMI, only the overweight showed an ageing-associated decrease in intrinsic strength. No ageing changes were significant with the population grouped by adiposity. Specific force was not affected by ageing in the pooled population nor where the population was grouped either by BMI or adiposity.

### The effect of BMI and adiposity on muscle contractile characteristics

The classification of obesity in the majority of previous studies investigating the effect of high levels of adiposity on muscle structure and function has been through BMI (Delmonico et al. 2009; Maffiuletti et al. 2008; Maffiuletti et al. 2007; Rolland et al. 2004; Hulens et al. 2002; Hulens et al. 2001). However, BMI does not distinguish between fat, lean, and bone mass in the calculation of obesity and instead utilises an individual's body mass relative to their height. We argued that this had the potential to conceal the true effect that differing levels of adiposity may have on skeletal muscle properties (Rothman 2008). Therefore, the results of the current study were categorised by both BMI and body fat percentage to determine whether one or the other obesity classification strategy would be the most powerful in distinguishing between the populations (Rolland et al. 2009; Baumgartner et al. 2004).

The two obesity classifications (i.e., by BMI or adiposity) provided comparable conclusions with regard to the reported magnitude of group differences in muscle contractile characteristics between obese and non-obese classes. This single conclusion supports the hypothesis that obesity does indeed load the antigravity musculature in a manner similar to simulated hypergravity (Bosco et al. 1986; Klentrou et al. 2007) and/or resistance training. Indeed our data shows an increase in both nMVC and muscle volume in the adult, young obese individuals. For instance, if primarily focusing on obesity effect on nMVC (net total muscle strength), there was a significant greater maximum torque whether participants were classified obese by BMI (+29%) or adiposity (+27%). Our results concur with previous research findings of +12% (Hulens et al. 2001) and +16.3% (Maffiuletti et al. 2007) significantly greater absolute knee extensor torque in obese compared with normal weight individuals. However, with the aforementioned two studies, the recorded maximum torque was not corrected for agonist muscle activation or antagonist co-activation, leading to an underestimation of the true torque of the obese individuals. Indeed, young obese adults have been demonstrated to have significantly lower (92% vs. 85%) agonist muscle activation capacity compared to age-matched normal weight counterparts (Chapter 2).

Similarly, obesity was associated with increased GM muscle volume when compared to normal weight (+71%) and ordinary adipose (+60%) classified females. Arguably, this further supports the idea that obesity in the young adult, potentially loads skeletal muscle similar to progressive resistance training (Erskine et al. 2010b). This theorem has been supported by Lafortuna et al. (2013) who demonstrated a positive association between adiposity and lower limb skeletal muscle volume using computed tomography in adult females. However, no other study, prior to our present work, had accurately quantified the musculature involved in the specific joint movement of interest, through accounting for the volume and architecture of said musculature.

While the above two parameters (i.e., nMVC and muscle volume) suggest a positive impact of obesity, when normalising maximum torque to the individual's muscle volume (intrinsic strength), a maladaptation of the skeletal musculature to obesity becomes evident. This is illustrated both when individuals were classified obese through BMI (-26% lower intrinsic strength) and adiposity (-11% lower intrinsic strength). These results suggest that at the whole muscle level, young adult obese individuals irrespective of obesity classification are at a disadvantage

relative to normal weight individuals. Interestingly, when comparing these results to previous work, there appears to be conflicting reports within the literature. On one hand, some researchers (Hulens et al. 2001) reported MVC knee joint torque normalised to total fat-free mass to be 6-7% significantly lower in obese females. Those authors hypothesised lower agonist muscle activation as being the physiological basis of this obesity effect. However, other authors (Maffiuletti et al. 2007) reported the differences between obese and non-obese muscles to disappear when torque was normalised to total fat-free mass. Arguably, muscle volume is a more accurate measure of muscle size than total fat-free mass (Akagi et al. 2009), hence direct comparison of the results from the literature is meaningless, potentially explaining the conflicting reports.

While normalising nMVC to muscle volume gives an accurate depiction of the whole muscle level features (i.e., the intrinsic strength), skeletal muscle specific force describes characteristics at fascicle level. The calculation of skeletal muscle specific force accounts for the physiological and biomechanical determinants of a muscles force generating capacity (Maganaris et al. 2001). The fascicle level results from our current study present a non-significant trend with the above-described whole muscle level findings. Indeed, the obese young females classified by both BMI and adiposity demonstrated lower (-17% and -15%, respectively (p=n.s.) for each classification method) skeletal muscle specific force compared to normal weight individuals. Since this is the first study to control for the neural, morphological, and biomechanical factors in force generation, there are no data at a fascicle level to compare our data against. Nonetheless, the underlying mechanism may potentially be linked to higher levels of inflammation often measured in obese individuals (Hotamisligil et al. 1995) and an increase in fatty infiltration within the muscle (Hilton et al. 2008), both likely to lower the intrinsic and specific strength potential of the obese skeletal musculature.

**Impact of BMI and/or adiposity on the magnitude of ageing-related sarcopenia and asthenia**

When rate of ageing was determined by populations for changes in muscle volume, nMVC, intrinsic strength, and specific force, the fastest changes were seen with muscle volume and nMVC (as seen in Figure 4.5). However, it was apparent that the greatest effect of combined adiposity and ageing was seen in the rate of loss of muscle volume (Figure 4.5.A and 4.5.C). One of the mechanisms

that can underpin the faster loss of muscle mass may be the cumulative effect of higher inflammation observed in both obese and elderly individuals (Cesari et al. 2004; Degens 2010; Park et al. 2005; Schrager et al. 2007) coupled with a lower anabolic profile both in old age (Bucci et al. 2013) and obesity (Frost et al. 2003; Galli et al. 2012). This enhanced susceptibility to sarcopenia in the obese, emphasises the deleterious impact of this condition, hence highlighting the importance of maintaining a healthy body composition.

The effect of ageing on nMVC was significant in old subject groups. However, there were no significant differences between the slopes of the regressions between obesity categories (see Figure 4.5.B and 4.5.D). The importance of this finding stems from a further exacerbation of an existing relatively low strength to body mass ratio in obese individuals (Blimkie et al. 1990; Lafortuna et al. 2005; Maffiuletti et al. 2008; Maffiuletti et al. 2007; Zoico et al. 2004), thereby rendering daily functional capacity even more compromised, with the ability to carry out tasks such as rising from a chair or squatting deep to reach items on the floor for instance, quicker to lose. Arguably, a more detailed description of decade-by-decade changes on sensitivity to adiposity would be warranted in future studies, as would the additional consideration of ethnicity.

Interestingly, in the current study population, the effect of ageing on either intrinsic strength or specific force (when categorised by BMI or adiposity) was as expected, as no significant changes were observed. Notably, however, ageing was associated with a significant decrement in intrinsic strength in the pooled population. Our data partially support previous reports that have shown specific force to decrease with age (Morse et al. 2005b). Interestingly, resistance training has been shown to increase specific force in the elderly (Morse et al. 2007). Therefore, assuming the additional fat mass seen in obesity chronically loads the skeletal muscle to a degree similar to resistance training, together with the extended proportion of their lifespan as obese individuals (Abdullah et al. 2011), may explain the lack of deleterious ageing-related changes in intrinsic strength and muscle specific force, even in this population. Potentially such an effect would be mediated via a shift in muscle fibre-type composition toward type II fibres. Indeed such fibre-type composition bias has previously been reported in obese individuals (Kriketos et al. 1997).

The lack of ageing-related decrease in three out of four BMI or adiposity subgroups in the current population may also be indicative of a healthy older population, as demonstrated through their health questionnaire data.

## Conclusion

This study demonstrates for the first time that at the whole muscle level, high BMI or adiposity categories of obesity are associated with significantly lower skeletal muscle contractile capacity in young adults. Interestingly, the ageing effect on obese individuals classified by both BMI and adiposity was foremost observed through the loss of muscle tissue content as well as total muscle strength. The dissociation in the ageing-related rate of changes in the BMI and/or adiposity categories meant that in the presence of obesity, ageing did not lower skeletal muscle intrinsic strength and/or muscle specific force. While this latter finding warrants further investigations, our results suggest that obesity even where individuals are recreationally active (as in the present study sample), should be targeted using therapies aimed at minimising sarcopenia and asthenia in later life.

# Chapter 5: The Continuum of Adiposity and its Effect on Skeletal Muscle Size, Structure and Function in Untrained Young versus Old Males

Data presented in: Tomlinson DJ, Erskine RM, Morse CI, Winwood K, Onambele-Pearson GL. The Continuum of Adiposity and its Effect on Skeletal Muscle Size, Structure and Function in Untrained Young versus Old Males. Poster session presented at the Physiological Society Meeting (Obesity: A Physiological Perspective), Newcastle (2014).

## Abstract

Obesity has been shown to have a loading effect on the anti-gravity skeletal muscles. However, the ageing of skeletal muscle functional and structural characteristics of obese male individuals has yet to be categorised. This study aimed to investigate the effect that the continuum of adiposity has upon skeletal muscle characteristics in young as opposed to older men. Thirty-four untrained healthy males categorised by age into young (Y) (mean ± SD: 25.3 ± 9.6 yrs) versus old (O) (68.7 ± 7.2 yrs), were assessed for body composition using dual energy x-ray absorptiometry, gastrocnemius medialis (GM) muscle volume (V) using B-mode ultrasonography, plantar flexion net maximum voluntary contraction (nMVC) correcting for both agonist muscle activation and antagonist co-contraction using an isokinetic dynamometer, intrinsic strength (nMVC/V) and GM skeletal muscle specific force (SF). BMI was positively associated with nMVC (Y r=0.760; p=0.002; O r=0.538; p=0.021) and V (Y r=0.762; p=0.002; O r=0.471; p=0.049). BMI correlation against GM SF was positive in the younger cohort (Y r=0.594; p=0.042) but not the old (O r=0.14; p>0.05), so that the difference between the two slopes was statistically significant (Y vs. O being 0.879 $N/cm^2$/BMI vs. 0.145 $N/cm^2$/BMI; Student's *t*-statistic 2.05, p<0.05). Total body adiposity elicited similar positive associations with nMVC (Y r=0.521; p=0.056; O r=0.585; p=0.011) and V (Y r=0.708; p=0.005; O r=0.548; p=0.019). Interestingly the rate of deleterious change with increased ageing, implied through regression slopes, in terms of both intrinsic strength (-0.639 $Ncm/cm^3$/year p=0.013) and GM SF (-0.373 $N/cm^2$/year; p=0.013) was significantly steeper in the obese (i.e. high BMI). Similarly, the rate of deleterious change with increased ageing in terms of both GM V (-1.577$cm^3$/year; p<0.05) and GM SF (-0.183 $N/cm^2$/year; p<0.05) was found to be faster in the highly adipose.

This study demonstrates that whilst high BMI (in spite of high adiposity) has a positive loading effect on absolute torque and muscle volume in young males, in older males, presence of obesity is detrimental to skeletal muscle function. Specifically the implied rate of ageing, at the fascicular level, suggests that the endocrine effects of combined adiposity and ageing may be additive. This effect would override the mechanical loading of chronic high loads and hence impede the physiological pathways responsible for muscle function optimisation.

## Introduction

There appears to be a limited amount of research into the effect obesity has upon skeletal muscle structure and function in adult men. The most comprehensive work appears to be reported in adolescent males (Abdelmoula et al. 2012; Blimkie et al. 1990; Maffiuletti et al. 2008). However, within this age classification discrepancies have been reported on the effect obesity has upon muscle strength, as Blimkie *et al.* (1990) reported no significant differences in absolute torque and absolute torque normalised to muscle anatomical cross sectional area (ACSA) between normal weight and obese adolescent males. In comparison, Abdelmoula *et al.* (2012) reported the obese adolescent males to have both significantly higher absolute torque and when normalised to estimated thigh muscle mass. These potential discrepancies between studies could be explained by the lack of control of physical activity status (Martinez-Gomez et al. 2011) and potential differences in the classification of muscle mass content. In an adult population Maffiuletti *et al.* (2007) revealed obese males to have 20% higher absolute knee extension maximum voluntary contraction (MVC) torque, yet 32% lower knee extensor MVC torque when normalised to body mass. However, when MVC torque was normalised to total body fat free mass all-significant differences were removed. The problem with normalising MVC torque to total fat free mass is the risk of masking the true effect obesity has upon normalised muscle torque. Furthermore, ACSA potentially underestimates the physiological cross sectional area (PCSA) of a pennate muscle (Alexander and Vernon 1975). PCSA is defined by an individual's muscle volume divided by its fascicle length and is directly proportionate to maximal muscle torque (Fukunaga et al. 2001). Therefore the use of total fat free mass however, could potentially exacerbate the differences further due to assumption total muscle mass equates to the number of parallel-aligned sarcomeres (i.e. the main determinant of maximum force) in the contracting muscle.

In the previous chapters, extensive and comprehensive research has examined the effect of adiposity on skeletal muscle structure and function in a young and old adult female population (Chapters 2-4). Chapter 2 reported absolute MVC plantar flexor (PF) torque corrected for agonist activation and co-activation normalised to body mass to be significantly lower in both young and old adult females. However in the older female cohort, no between group differences were reported in absolute PF MVC torque, whilst the younger obese females had

20% higher absolute PF MVC torque than normal weight counterparts. In addition, Chapter 3 reported both increasing BMI and total adiposity to elicit a loading effect on the PCSA of the gastrocnemius medialis (GM). This study concluded that in a female population, adiposity in the young is associated with higher MVC torque, consistent with the adaptations observed when loading through hyper-gravity training (Bosco 1985; Bosco et al. 1986; Bosco et al. 1984). However, it is not known if this association between adiposity and MVC torque is also present in an adult male population. Lafortuna et al. (2013) reported total body adiposity to have a loading effect on total muscle volume of the lower limb using computed tomography in both males and females. However, within this study (Lafortuna et al. 2013), the response of loading from differing levels of total body adiposity differed between genders, as the males had a greater hypertrophic response to the loading of high levels of adiposity. The potential explanations for these differences may potentially be explained by the 10-15 fold higher levels of testosterone reported in males (Tipton 2001). The hormone testosterone is known to increase protein synthesis, thus could potentially create a greater anabolic adaptation created by the loading of the lower limbs through carrying high overall mass. The differing gravity induced loading muscular responses to obesity between genders is further supported when comparing the effect obesity classification had upon absolute knee extensor isotonic strength between adult male and females (Lafortuna et al. 2005). The sex difference in maximal strength was exaggerated in the obese males, whereby the gravity induced loading results in greater sex differences in fat free mass (Lafortuna et al. 2005). However, a more stringent measure of contractile strength that has yet to be examined in an obese male population is skeletal muscle specific force. Skeletal muscle specific force accounts for the physiological and biomechanical determinants of maximal strength capacity (as previously detailed within chapter 1), such as moment arm length, agonist muscle activation, antagonist co-activation, PCSA and skeletal muscle fascicle pennation angle (Erskine et al. 2009; Maganaris et al. 2001; Reeves et al. 2004b). Specific force has been previously shown to be decreased with ageing, yet has been increased following progressive resistance training (Morse et al. 2007; Reeves et al. 2004b). Interestingly, chapter 4 details there to be no decrease in specific force with ageing in an obese female cohort ranging from 18-80 years old. However, within chapter 4, the young female cohort were reported to have significantly lower intrinsic strength (absolute torque relative to muscle volume) at a whole muscle level in the obese/high adipose female cohort

compared to normal weight individuals. This type of observation however has not been made in a male population and provides the rationale for differentiating the effect of high adiposity on the antigravity musculature by sex.

The aim of the present study was to investigate the effect the continuum of adiposity has upon skeletal muscle characteristics, in a male population. The second aim was to determine whether the effects of ageing and adiposity are in fact additive on skeletal muscle specific force at both whole body and fascicle levels. It was hypothesised that: (1) The slopes of the regressions of skeletal muscle MVC torque and muscle volume vs. BMI/adiposity in the old would be lower when compared to young individuals. (2) The deleterious impact of high adiposity on skeletal muscle specific force, would be worse in the older individuals than their younger counterparts; (3) based on previous chapters results, the rate of change seen with ageing (decrease in muscle contractile capacity) would be faster in the presence of obesity.

## Methods

**Participants**:

Thirty-four untrained males volunteered to take part in this study (Table 5.1) and were categorised by age into either Young (Y; 18-49yrs) or Old (O; 50-80yrs). Participants were then sub-categorised into three body mass index classifications (BMI – Body Mass (kg)/Stature$^2$ (m)) into Normal (BMI 18.5-24.9), Overweight (BMI 25.0-29.9) and Obese (BMI > 30.0). Participants were also categorised as ordinary-adipose <28%) vs. high-adipose by body fat percentage (≥28%) following classification from a previous study (Baumgartner et al. 2004). The exclusion criteria were issues with lower limb muscles/joints affecting mobility or ability to exert MVC torque (e.g. Osteoarthritis (Alnahdi et al. 2012)). Physical activity status was screened by questionnaire and participants were excluded if they self-reported as habitually undertaking structured exercise for more than 3 hours per week and any form of resistance training. The elderly cohort recruited for this study included only community dwelling and independently living individuals.

Participants gave written-informed consent prior to undertaking any assessment. All the procedures in this study had approval from the Manchester Metropolitan University Ethics committee and conformed to the standards set by the latest revision (2012-2013) of the Declaration of Helsinki.

**Table 5.1.** Descriptive variables for obesity classification by adiposity in both the combined young (upper section) and old (lower section) age classifications. Data are presented as Mean ± SD.

| | Ordinary-Adipose (n=15) | High-Adipose (n=19) | Obesity |
|---|---|---|---|
| | Mean±SD | Mean±SD | Effect |
| **Age Range (yrs)** | 18-80 | 18-80 | |
| **BMI (kg/m$^2$)** | 24.3±3.2 | 28.9±4.0 | p=0.001 |
| **Body mass (kg)** | 76.8±11.2 | 86.3±10.2 | p=0.015 |
| **Body fat %** | 22.9±4.0 | 32.1±4.4 | p<0.001 |
| **Fat mass (kg)** | 17.2±4.9 | 27.1±6.1 | p<0.001 |
| **Lean mass (kg)** | 54.1±6.5 | 53.6±5.3 | p=0.819 |
| **AG Ratio** | 1.03±0.1 | 1.21±0.2 | p=0.001 |

## Protocol order:

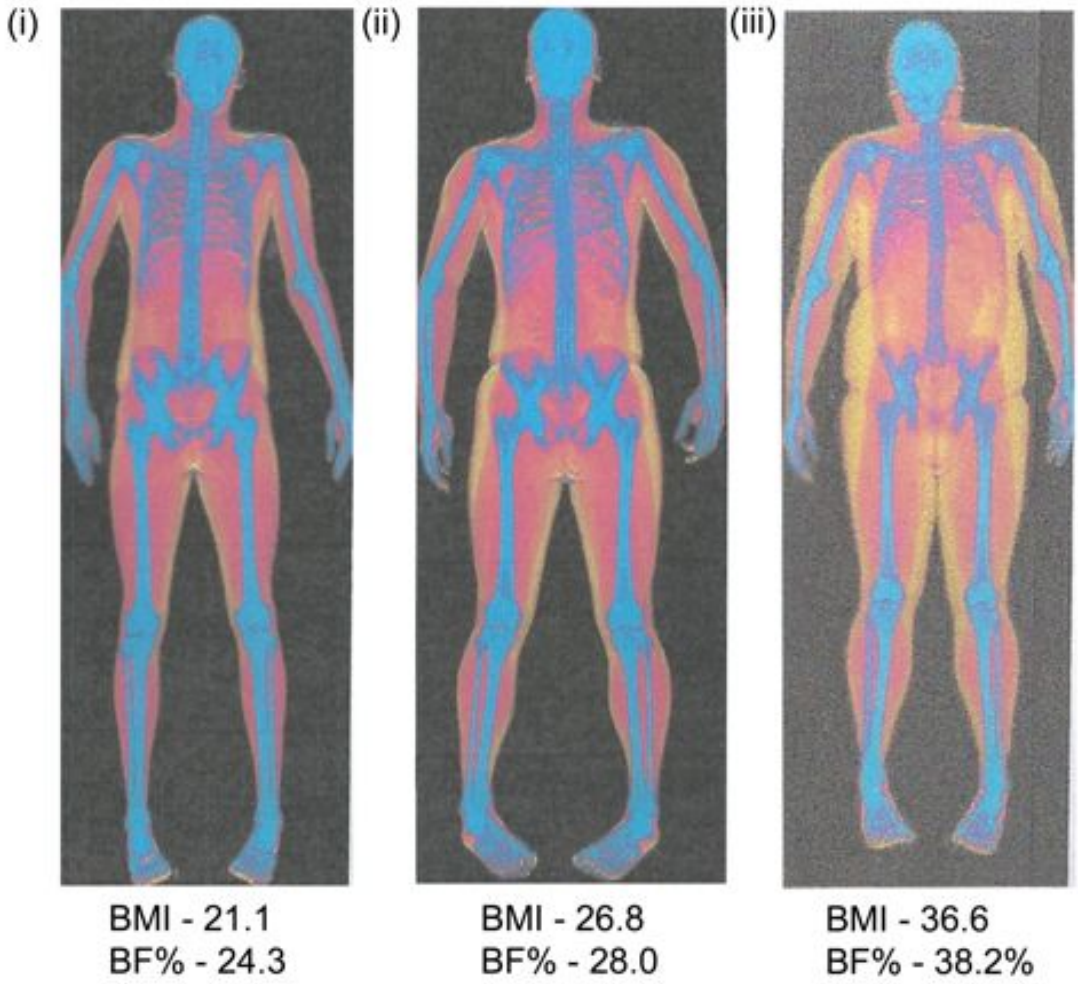
Participants attended the laboratory for testing on two occasions. During the first visit, anthropometric measurements (DEXA, Stature, Mass) were collected, and familiarisation with the MVC protocols took place. During the second visit participants gastrocnemius medialis muscle volume and architecture data were collected alongside the muscle contractile properties test protocols proper.

## Body Composition Measure:

Body Composition (body fat percentage, lean muscle and bone) was ascertained using a DEXA scanner (Hologic Discovery: Vertec Scientific Ltd, UK) following a period of overnight fasting for 12 hours (Figure 5.1). Participants lay in a supine position, avoiding any contact between the trunk and the appendicular mass during a 7 min scanning procedure (effective dose 8 μSv). Segmental analysis of the whole body scan using the Hologic APEX software (version 3.3) provided quantification for appendicular skeletal muscle mass (ASM) used in the classification of sarcopenia and additionally participants' android to gynoid ratio (AG). The android region was classified as the area between the mid-point of the lumber spine to the top of the pelvis, whilst the gynoid region was classified as the area between the head of the femur and mid-thigh. ASM was calculated as the

total muscle mass of both the upper and lower limbs. The ASM index was then calculated using the following calculation - ASM/height$^2$ (kg/m$^2$). Sarcopenia was then defined as participants, who had an ASM index below 7.26 kg/m$^2$ as previously quantified by Baumgartner *et al*. (2004).

**Figure 5.1.** Representative dual-energy x-ray absorptiometry scans of a (i) normal weight male, (ii) overweight male and (iii) obese male. Colour key: Blue for bone; Red for Lean tissue, Yellow for adipose tissue.




**Muscle Strength Measurement:**

MVC torque during both ankle PF and Dorsiflexion (DF) was measured in the self-reported dominant limb (described as the leg they would preferentially kick a ball with) using an isokinetic dynamometer (Cybex Norm, Cybex International, New York, NY). Participants were seated with their hip angle positioned at 85°, dominant leg extended with the foot secured to the footplate of the dynamometer and strapped using inextensible straps at the hip, distal thigh and chest to reduce

extraneous movements. Prior to maximal contractions, participants were re-familiarised with the protocol. Following this familiarisation protocol, participants conducted a series of 5 sub-maximal isometric contractions with their ankle positioned at 0° (anatomically neutral), starting at a self-perceived 50% maximal exertion, increasing in intensity to ensure the participant would be warmed up prior to maximal exertion.

During the MVC test proper, participants were asked to conduct two rapid isometric PF and DF MVCs with their ankle positioned at 0°, each lasting 3-4 seconds. The highest recorded PF and DF MVC by the participant was utilised as their true MVC. It is notable that MVCs were repeated if there was > 10% difference between MVCs to ensure true MVC was obtained. The PF MVC was then corrected for agonist muscle activation using the interpolated twitch technique (Morse et al. 2004; Pearson and Onambele 2006) and antagonist co-contraction of the tibialis anterior (TA) using surface electromyography (EMG), so that the resultant data would be classified as net MVC (nMVC). The methodology for calculation of voluntary agonist activation level) and antagonist co-contraction is detailed in more depth below (please see also chapter 2).

**Muscle Activation**

PF agonist muscle activation was estimated using the interpolated twitch technique (Morse et al. 2004; Pearson and Onambele 2006). Briefly, percutaneous stimuli (DSVDigitimer Stimulator; Digitimer, Herts., UK) were applied to the gastrocnemius using rubber stimulation pads (50mm x 100mm; American Imex; Irvin, CA, USA). The two stimulation pads were placed transversely distal to the popliteal crease and myotendinous junction of the gastrocnemius lateralis. The amplitude of the stimulus was determined prior to interpolation whilst the participant was in a relaxed state; administering doublet twitches delivered with a pulse width of 200 $\mu$s, and an interpulse interval of 10 ms to increase the signal to noise ratio. Doublet twitches started from 50 mA, with subsequent 50-100 mA increments, until no further increase in twitch torque was elicited. The assessed supramaximal doublets (i.e. the stimulus intensity above which no further increase in twitch torque was observed with increased stimulus intensity) were superimposed during a maximal PF MVC. The calculation used to establish muscle activation is shown below:

1-(Superimposed torque increase/resting superimposed torque) × 100 = muscle activation (%)

**Antagonist Muscle Co-contraction**

Surface EMG using pre-gelled unipolar Ag-AgCl electrodes (Medicost, Denmark) was used to assess muscle co-contraction of the TA during PF MVC. Two electrodes (skin contact size 30mm x 22mm) were placed proximally at one third of the tibialis anterior muscle length, mid muscle belly, with a 1-2 mm gap separating each electrode. A reference electrode (Medicost, Denmark) was placed on the head of the fibula. Raw EMG was then recorded at 2000Hz, with band pass filter set at 10-500Hz, and notch at 50 Hz.

Muscle co-contraction (%) was calculated utilising the raw EMG signal computed as root mean square (RMS) 500 milliseconds either side of instantaneous peak torque of the tibialis anterior during PF MVC divided by EMG during DF MVC. Co-contraction torque (Nm) was the product of % co-contraction and maximal DF torque. This method assumes that the DF EMG/Torque relationship is linear (Maganaris et al. 1998).

**Muscle Volume**

GM muscle volume was calculated using the truncated cone method through the construction of several ACSAs taken at discrete muscle sites (25, 50, and 75% of length) using B-mode ultrasonography (AU5 Harmonic, Esaote Biomedica, Genoa, Italy). Participants lay in the prone position with their ankle positioned in neutral (90 degrees angle, referred to here as 0 degrees). B-mode ultrasonography was then used to ascertain the origin and insertion of the GM, where the aforementioned discrete muscle sites were marked from the medial to lateral border of the GM. Thin strips (5 mm long, 2 mm wide) of micropore tape (3M, Bracknell, UK) were placed 3-4cm apart along each of the three medial to lateral border lines along the discrete muscle sites, always crossing the border line at right angles (see Figure 3.2 of chapter 3). The micropore tape was utilised as echo-absorptive markers to help construct the ACSAs at the three muscle sites with the use of image editing software (Adobe Photoshop; Version 10). During recording of the ACSAs the ultrasound probe (7.5 MHz linear array probe, 38 mm wide) was held transversely and perpendicular to the GM on its medial border and moved along a designated marked pathway to its lateral border to ensure the

probe was kept perpendicular to the GM during the whole scanning procedure. The probe was moved steadily across the leg with constant light pressure to avoid compression of the muscle during scanning. This procedure was repeated twice at each discrete muscle site for reliability purposes. Multiple transverse images at each muscle site were reconstructed with the editing software using the micropore tape as echo absorptive markers in combination with anatomical landmarks within the GM muscle (see Figure 3.3 of chapter 3). Following construction of the three individual ACSAs, the areas of the complete ACSAs were measured using the analysis software ImageJ (1.45s; National Institutes of Health, Bethesda, Maryland. In order to calculate the total muscle volume, an area of 0.5cm$^2$ was assumed as a standard for 0 and 100% of GM muscle length. Muscle volume was then calculated using the truncated cone method constructing 4 cones:

Single Cone Volume= (⅓ x h) x π x (R1$^2$ + R1) x (R2$^2$ + R2)
Where R1 = radius of the base ACSA; R2 = radius of the top ACSA; h = distance between segments; R = √(ACSA/π), where π = 3.142

PCSA was then subsequently calculated using the ratio between GM fascicle length to muscle volume, i.e. PCSA = GM muscle volume (cm$^3$)/ GM fascicle length (cm).

**Muscle Architecture**

Muscle architecture of the GM was measured using B-mode ultrasonography (AU5 Harmonic, Esaote Biomedica, Genoa, Italy) at both rest and during a graded maximal MVC over 6 seconds. Participants were seated in an isokinetic dynamometer (Cybex Norm, Cybex International, New York, NY) as per the Muscle Strength Measurement protocol. Resting fascicle pennation angle (FPA) and fascicle length (Lf) were measured with the ultrasound probe positioned at 50% of the GM muscle length, at mid muscle belly in the sagittal plane. Participants were then asked to perform a ramped PF MVC over 6 seconds, where the change in both FPA and Lf were also recorded on the capturing software (Adobe Premier pro Version 6, Adobe Systems Software, Ireland). Both resting and maximal images (see Figure 3.1 of chapter 3) (the latter synchronised with torque outputs using a square wave signal generator) were extrapolated from the capturing software and analysed using ImageJ (1.45s; National Institutes of

Health, Bethesda, Maryland). Three fascicles defined from the deep to the superficial aponeurosis were analysed and the mean value of Lf and FPA were recorded. If needed, linear extrapolation was used on fascicles that extended off the edge of the screen by extending the superficial and deep aponeurosis of the GM. The errors in extrapolation of skeletal muscle fascicle lengths have previously reported to be between 2-7% (Muraoka et al. 2001; Finni et al. 2003). Both the Lf and FPA taken during MVC were used in the calculation of skeletal muscle specific force.

**Achilles Tendon Moment Arm**

Tendon excursion using B-mode ultrasonography (AU5 Harmonic, Esaote Biomedica, Genoa, Italy) was used to calculate the Achilles tendon moment arm length (Maganaris et al. 2000; Ito et al. 2000). Participants were seated in the isokinetic chair (Cybex Norm, Cybex International, New York, NY) following the experimental set up undertaken during MVC's. Prior to commencement of the protocol, the insertion of the GM muscle to the Achilles tendon was anatomically marked on the limb using a thin strip (2mm) of micropore tape (3M, Bracknell, UK) as an echo-absorptive reflective marker during recording. The ultrasound probe (7.5 MHz linear array probe, 38 mm wide) was then positioned across the muscle-tendon junction (MTJ) of the GM as denoted by the micropore tape. During the recording the ankle of the participant was passively rotated between 10° to -5° PF at a constant velocity of 1 °/s. The passive movement was recorded for at least 3 PF and DF rotations and was synchronised with torque outputs using a square wave signal generator to distinguish joint position on the capturing software.

The displacement of the MTJ of the GM between 10° PF to -5° PF was calculated using the micropore tape as a distance marker using analysis software (ImageJ 1.45s; National Institutes of Health, Bethesda, Maryland). The Achilles tendon moment arm length at 0° was then calculated using the displacement of the MTJ divided by the displacement in the ankle angle during passive movement through the participants voluntary range of motion. The coefficient of variation of the tendon excursion method of the Achilles tendon using ultrasound has previously been reported to be 4.5-9.7% (Fath et al. 2010). In addition, the aforementioned method has been significantly correlated with the centre of rotation method using MR scans (Fath et al. 2010).

$MA_{0^\circ}$ = displacement MTJ ÷ change in ankle angle

**Calculation of GM Specific Force**

This involved several steps including the assessment of tendon force and fascicle force in order to calculate GM specific force.

*Tendon Force*- Achilles Tendon force (F) at 0° was calculated as PF nMVC divided by the Achilles tendon moment arm length. The contribution of the GM muscle to PF maximal force was calculated, assuming this muscle to contribute 25% total ankle PF MVC force (Fukunaga et al. 1992).

$F_{GM}$ = F x 0.25, where F = nMVC ÷ $MA_{0^\circ}$

GM Fascicle Force- Following the computation of the GM muscle force ($F_{GM}$), fascicle force was calculated by dividing $F_{GM}$ by the cosine of GM FPA:

GM $F_{fasc}$ = $F_{GM}$ ÷ Cos FPA

GM specific force was calculated by dividing the $F_{fasc}$ by GM PCSA (Alexander and Vernon 1975; Fukunaga et al. 1996; Reeves et al. 2004b).

GM SF = GM $F_{fasc}$ ÷ PCSA

**Reliability**

The reliability in the measurement of both muscle architectural characteristics (muscle fascicle pennation angle and length) and GM ACSA were undertaken in 10 participants (Y = 5; O = 5; BMI range = 17.6-36.7) on two separate days (separated by at least 48hrs) by the same investigator.

The Intra Class Coefficients for all the measurements were high and significant for all of the assessment techniques (muscle fascicle pennation angle rest - 0.997, muscle fascicle pennation angle max - 0.997, muscle fascicle length rest - 0.996, muscle fascicle length max 0.993, GM ACSA 25% length - 0.998, GM ACSA 50% length - 0.999, GM ACSA 75% length - 0.998). The measurements of the ACSAs used in the construction of muscle volume are both reliable and have

previously been demonstrated to be in strong agreement with GM ACSA obtained using MRI (Reeves et al. 2004a).

### Statistical analyses

Statistical analyses were carried out using SPSS (Version 19, SPSS Inc., Chicago Illinois). To determine parametricity, Shapiro-Wilk (normal distribution) and Levene's tests (homogeneity of variance) were utilised. Linear regressions and Pearson's or Spearman moment correlations, were then used to describe the relationships and the degree of association, between age and muscle volume, nMVC, nMVC relative to muscle volume (GM intrinsic strength) and GM specific force. Comparisons of the regression coefficients and slopes were conducted using z-transformations and the Student's *t*-statistic. Data are reported as mean ± SD and statistical significance was accepted when $p \leq 0.05$.

## Results

### Body Composition

Table 5.1 displays the descriptive study population characteristics irrespective of age, categorised by Body fat % as either normal adipose or high adipose. As expected, there were significant differences between the categorised groups for BMI (p=0.001), body mass (p=0.015), body fat % (p<0.001) and total adiposity (p<0.001).

### Skeletal muscle characteristics

*BMI and muscle contractile characteristics*

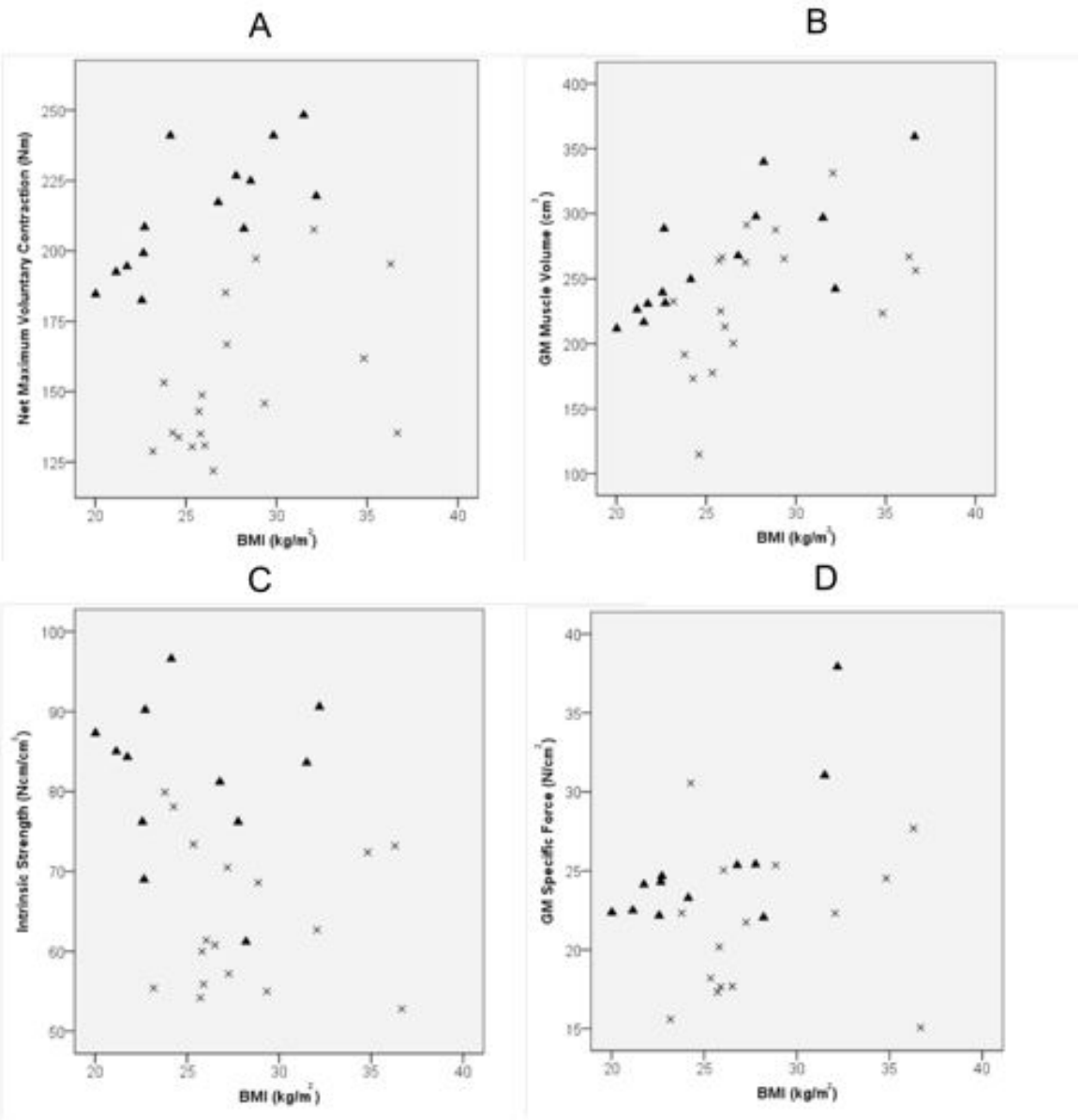
Figure 5.2 and Table 5.2 demonstrate the associations between the participants' BMI and nMVC, GM muscle volume, GM intrinsic strength and GM specific force in both Y and O males. Both Y and O males demonstrated that higher BMI was positively associated with nMVC (Y r=0.760, p=0.002; O r=0.538, p=0.021). This response was mirrored in the effect BMI had upon muscle volume, as demonstrated by a significant positive association with rising BMI and muscle volume (Y r=0.762, p=0.002; O r=0.471, p=0.049). No significant differences were reported between the slope of regressions between Y and O individuals for nMVC

and GM volume (as seen in Table 5.2). In addition, there were no significant associations between BMI and GM intrinsic strength (p>0.05). However, the slopes of the regressions between BMI on GM specific force differed between the Y and O cohort (Y 0.878 N/cm$^2$/kg vs. O 0.145 N/cm$^2$/kg; Student's *t*-statistic 2.05; p<0.05); with the Y participants demonstrating a positive association with rising BMI and GM specific force (Y r=0.594; p=0.042), however the O individuals had a non-significant association between the same two parameters (O r=0.144 p=0.609).

**Table 5.2.** Pearson correlations, z transformation of r and Student's *t*-statistic between net maximum voluntary contraction (nMVC), gastrocnemius medialis (GM) muscle volume, GM intrinsic strength and GM specific force against BMI and adiposity in untrained young and old males (* P<0.05, ** P<0.01, *** P<0.001) (If Z > 1.96, p<0.05; Z > 2.58, p<0.01) (Student's *t*-statistic significance if *t* falls outside ± 1.96 p<0.05)

| | Young | | | Old | | | Correlation co-efficient | Ageing Effect |
|---|---|---|---|---|---|---|---|---|
| | n | r value | slope | n | r value | slope | Z-transformation of r | Student's *t*-statistic |
| nMVC vs. BMI | 14 | 0.76** | 4.02Nm/kg/m$^2$ | 18 | 0.54* | 3.08Nm/ kg/m$^2$ | 0.79 | 0.56 |
| Muscle Volume vs. BMI | 14 | 0.76** | 7.04cm$^3$/ kg/m$^2$ | 18 | 0.47* | 5.75cm$^3$/ kg/m$^2$ | 1.02 | 0.40 |
| Intrinsic Strength vs. BMI | 12 | 0.10 | -0.24Ncm/cm$^3$/ kg/m$^2$ | 17 | 0.04 | -0.08Ncm/cm$^3$/ kg/m$^2$ | 0.41 | -0.17 |
| Specific Force vs. BMI | 12 | 0.59* | 0.88N/cm$^2$/ kg/m$^2$ | 15 | 0.14 | 0.15N/cm$^2$/ kg/m$^2$ | 1.43 | 2.05* |
| nMVC vs Adiposity | 14 | 0.52 | 1.56Nm/kg | 18 | 0.59* | 1.61Nm/kg | -0.21 | -0.04 |
| Muscle Volume vs Adiposity | 14 | 0.71** | 3.64cm$^3$/kg | 18 | 0.55* | 4.22cm$^3$/kg | 0.55 | -0.30 |
| Intrinsic Strength vs Adiposity | 12 | 0.17 | -0.23Ncm/cm$^3$/kg | 17 | 0.23 | -0.30Ncm/cm$^3$/kg | -0.23 | 0.14 |
| Specific Force vs Adiposity | 12 | 0.31 | 0.41N/cm$^2$/kg | 15 | 0.06 | -0.04N/cm$^2$/kg | 1.01 | 1.94 |

**Figure 5.2.** Displays linear regressions on the impact of BMI on net maximum voluntary contraction (A), gastrocnemius medialis (GM) muscle volume (B), GM intrinsic strength (C) and GM specific force (D) categorised by age.
(Young =▲; Old = ×)

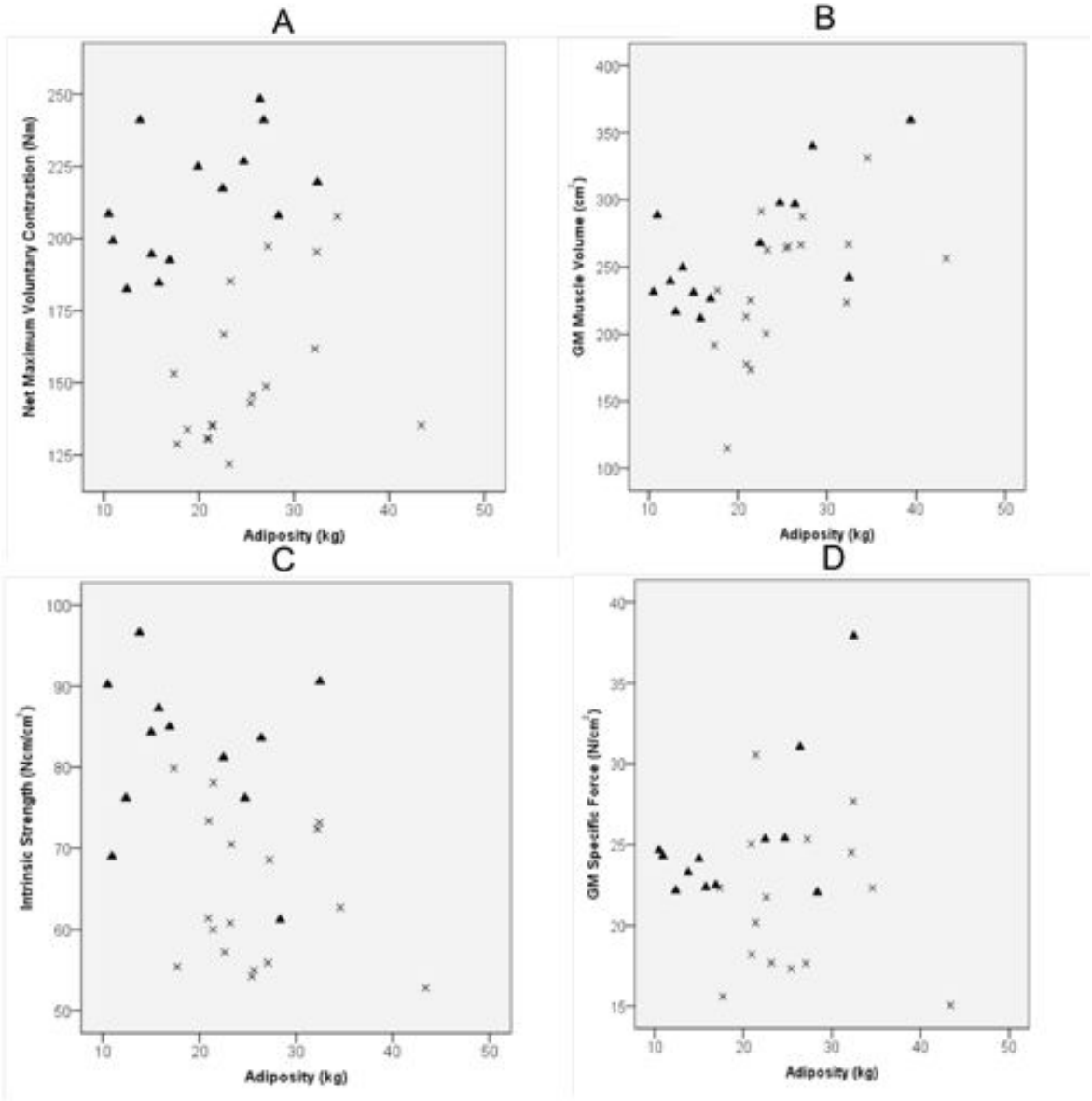



*Adiposity and muscle contractile characteristics*

Figure 5.3 and Table 5.2 demonstrate the associations between the participants' total body adiposity and nMVC, GM volume, intrinsic strength and GM specific force in both Y and O males. Only the O males demonstrated that higher adiposity was positively associated with PF nMVC (Y r=0.521, p=0.056; O r=0.585, p=0.011) in a similar manner to the effect of BMI. However, both Y and O males demonstrated that GM volume was positively associated with total adiposity (Y r=0.708; p=0.005; O r=0.548; p=0.019). No significant differences were

reported between the slope of regressions between young and old individuals for nMVC and GM volume (as seen in Table 5.2). In addition, there were no significant associations between higher total body adiposity and GM intrinsic strength (p>0.05). However different from the effect of BMI, total body adiposity had no significant effect on the slopes of GM specific force between the Y and O cohort (Y 0.408 N/cm$^2$/kg vs. O -0.004 N/cm$^2$/kg; Student's *t* statistic 1.94; p>0.05) and there was no-significant association in both Y and O individuals (Y r=0.308; p=0.331; O r=0.062; p=0.826).

**Figure 5.3.** Displays linear regressions on the impact of total adiposity on net maximum voluntary contraction (A), gastrocnemius medialis (GM) muscle volume (B), GM intrinsic strength (C) and GM specific force (D) categorised by age. (Young =▲; Old = ×)



*Degree of association between age and muscle size and/or strength by BMI and adiposity status*

Starting with males classified by their BMI, the slopes of the regression on the nMVC vs. ageing relationship implied the obese males (-1.25 Nm/year; p>0.05) to lose nMVC at a similar rate than their normal weight (-1.09 Nm/year; p=0.002) counterparts. However, the slope of the regressions indirectly implied the overweight males (-1.68 Nm/year; p=0.001) loss in nMVC torque occurs at a fast rate reaching the same strength level of their normal weight counterparts by the 8$^{th}$ decade (normal weight vs. obese, Student's *t*-statistic 0.18; p>0.05; overweight vs. obese, Student's *t*-statistic 0.49; p>0.05).

The GM volume vs. ageing relationship only revealed a significant negative association with overweight males (-1.59 cm$^3$/years; p=0.015). There were no significant differences in the slopes of the regressions between the three BMI classifications.

The intrinsic strength vs. ageing relationship, revealed an increase in the steepness of the slopes, comparing the overweight (-0.21 Ncm/cm$^3$/year; p=0.114) against the obese (-0.64 Ncm/cm$^3$/year; p=0.013) participants. These slopes were found to be significantly different (Student's *t*-statistic 2.25; p<0.05).

Similarly, the association between GM specific force vs. ageing revealed the steepness of the regression slope to increase, with increasing BMI classification. Thus, the steepness of slopes were -0.03 N/cm$^2$/year (p=0.543), -0.08 N/cm$^2$/year (p=0.140) and -0.37 N/cm$^2$/year (p=0.007) in the normal weight, overweight and obese BMI categories respectively. These differences in slopes were statistically significant (obese vs. normal weight, Student's *t*-statistic -4.51; p<0.05; obese vs. overweight, Student's *t*-statistic -5.13; p<0.05). Based on these slopes of the regression, it can be indirectly implied that the loss GM specific force with ageing was associated with faster than normal deleterious changes, when classified obese.

In addition, when males were classified by body fat percentage, the slopes of the regressions were steeper in the high adipose cohort (-1.58 Nm/year; p<0.001) in comparison to the normal adipose (-0.89 Nm/year; p=0.038) classified males. Thus, indirectly implying the high adipose males had a faster rate of loss in nMVC torque with increasing age. However, this difference in the slope was not statistically significant (Student's *t*-statistic 1.47; p>0.05) (Figure 5.4.A)

Similarly, there was a negative association between GM muscle volume and age in the high adipose cohort (r=-0.515; p=0.029). The slope of the regressions indirectly implied the high adipose (-1.58cm$^3$/year) cohort to lose muscle content at a greater rate between the second to the eighth decade than the normal adipose cohort (0.05cm$^3$/year). These differences in slopes were statistically significant (Student's *t*-statistic 2.03; p<0.05) (Figure 5.4.B). Based on these slopes, it can be indirectly implied that the loss of GM muscle volume with ageing was associated with faster than normal deleterious changes, when classification by body fat percentage.

There was a negative association between intrinsic strength and age in both high adipose (-0.31 Nm/cm$^3$/year; p=0.024) and normal adipose (-0.35 Nm/cm$^3$/year; p=0.018) cohorts. However, both the relationship and slopes of the regression were statistically similar between the normal and high adiposity groups (Table 5.3).

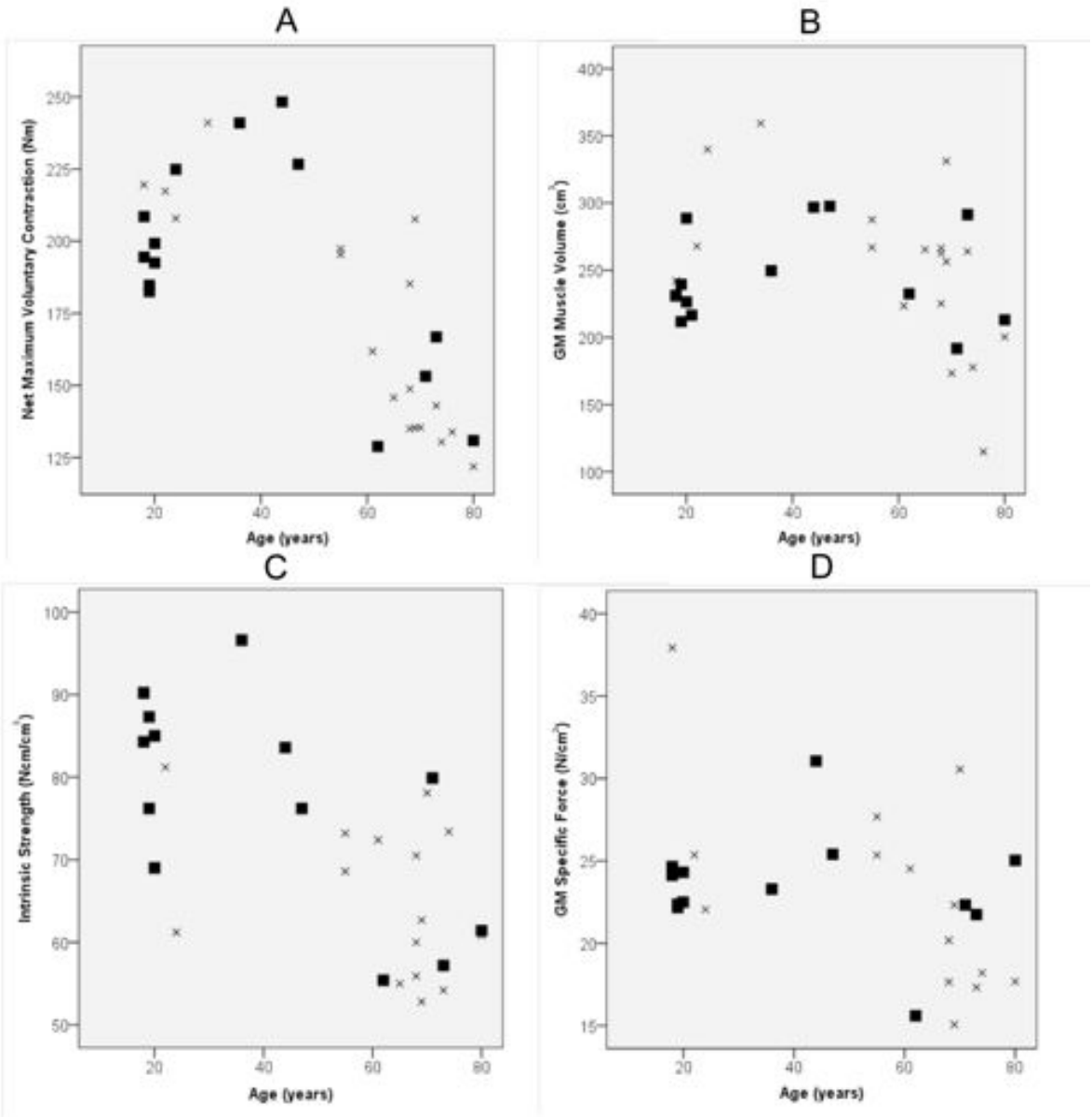
Unexpectedly, there was no association between GM specific force and age in the normal adipose cohort (-0.02 N/cm$^2$, p>0.05). However, the slope of the regression indirectly implied the high adipose cohort to lose greater GM specific force with increasing age (-0.18 N/cm$^2$, p=0.019). These differences in slopes of the regression were statistically significant (Student's *t*-statistic 2.03; p<0.05) (Figure 5.4.D). Similarly to when obesity was classed by BMI, the slopes of the regression indirectly implied that the loss GM specific force with ageing was associated with faster than normal deleterious changes, when classified as high adipose.

A Mann Whitney test revealed the O cohort to have significantly lower intrinsic strength (p<0.001) and GM skeletal muscle specific force (p=0.036).

**Table 5.3.** Pearson correlations, z-transformation of r and Student's *t*-statistic between nMVC, gastrocnemius medialis (GM) muscle volume, intrinsic strength and GM specific force against age in untrained normal and high adiposity males (* P<0.05, ** P<0.01, *** P<0.001) (If Z > 1.96, p<0.05; Z > 2.58, p<0.01) (Student's *t*-statistic significance if *t* falls outside ± 1.96 p<0.05)

| | Normal Adiposity | | | High Adiposity | | | Correlation co-efficient | Obesity Effect |
|---|---|---|---|---|---|---|---|---|
| | n | r value | slope | n | r value | slope | Z-transformation of r | Student's *t*-statistic |
| nMVC vs. Age | 14 | 0.59* | -0.89Nm/year | 18 | 0.87*** | -1.58Nm/year | -1.25 | 1.47 |
| Muscle Volume vs. Age | 14 | 0.03 | 0.05$cm^3$/year | 18 | 0.52* | -1.58$cm^3$/year | -2.05* | 2.03* |
| Intrinsic Strength vs. Age | 13 | 0.64* | -0.35Ncm/$cm^3$/year | 16 | 0.56* | -0.31Ncm/$cm^3$/year | 0.26 | -0.21 |
| Specific Force vs. Age | 13 | 0.15 | -0.02N/$cm^2$/year | 14 | 0.62* | -0.18N/$cm^2$/year | -1.48 | 2.03* |

**Figure 5.4.** Displays linear regressions on the impact of age on net maximum voluntary contraction (A), gastrocnemius medialis (GM) muscle volume (B), GM intrinsic strength (C) and GM specific force (D) categorised by body fat percentage. (Normal Adipose = ■ ; High Adipose = ×)




## Discussion

This study systematically demonstrated that the continuum of BMI is positively associated with nMVC and GM volume in both young and old untrained males. Interestingly at a fascicular level, an increase in BMI is positively associated with higher GM specific force in the young men but not the older males. The steepness in the slope of regressions between age and both intrinsic strength and GM specific force were observed to be higher in the obese. This would

suggest a greater age associated weakening of the PF muscle group in obese groups with ageing, thus supporting the first and second hypothesis.

Interestingly, the continuum of total body adiposity was only associated with nMVC in older males. However, muscle volume was positively associated with total body adiposity, in both young and old untrained males. Different to the effect of BMI, there was no association between total body adiposity and either intrinsic strength or GM specific force. The steepness in the slope of regressions between age and both nMVC and GM muscle volume were observed to be higher in the high adipose cohort. In addition, there were negative associations between age and intrinsic strength in both normal adipose and high adipose groups. Interestingly at a fascicular level, the slope of the regression between age and GM specific force indirectly implied that high adiposity was associated with faster than normal deleterious age related changes, of which are associated with sarcopenia (Morse et al. 2005b).

**The effect of BMI and adiposity on muscle contractile characteristics**

Classifications of obesity by both adiposity and BMI have both been shown to induce a loading effect on both skeletal muscle nMVC torque and GM muscle volume in Y untrained females (as seen in chapters 2-4). The results from this chapter partially concur with Chapter 4, as demonstrated by the continuum effect of both increasing adiposity and BMI increasing both nMVC and GM volume in untrained males. This study supports the hypothesis that increasing BMI and/or adiposity load the anti-gravity musculature in a manner similar to simulated hypergravity (Bosco 1985; Bosco et al. 1986; Bosco et al. 1984) and/or resistance training (Erskine et al. 2010b; Morse et al. 2007; Reeves et al. 2004b) in both young and old males. The increase in nMVC in conjunction with either increasing adiposity and/or BMI would support previous work conducted in an adult population demonstrating obese individuals having greater absolute strength (Hulens et al. 2001; Lafortuna et al. 2005; Maffiuletti et al. 2007). Interestingly this chapter demonstrated that both the Y and O males to significantly increase nMVC in conjunction with a rise in BMI. This response is not supported in an elderly female population, as chapter 4 revealed elderly obese females to not have significantly higher nMVC torque than their overweight, normal weight and underweight counter parts. The rationale for this hypothesis maybe partially due to

lower anabolic hormones (Tipton 2001) in the female cohort decreasing the plasticity of the skeletal muscle to adapt to the loading stimulus of obesity, thus explaining why differences were reported in this chapter. However, the precise mechanism in the adaptation to the mechanical load placed by excessive levels of adiposity evident in obesity warrants further investigations.

In conjunction with an increment in nMVC, GM muscle volume was shown to significantly increase with either increasing total body adiposity or BMI in both the young and old (Table 5.2). The mirroring in the rise in GM muscle volume accompanying the rise in nMVC partially explains the rise in nMVC torque, as demonstrated following progressive resistance training (Aagaard et al. 2001). The augmentation in muscle volume with both increasing adiposity or BMI finding is supported by Lafortuna *et al.* (2013) who demonstrated a positive association between adiposity and lower limb skeletal muscle volume measured with computed tomography in both adult males and females. The novelty of this chapter is the separation in the association by age, demonstrating that the loading effect of adiposity or BMI is similar between a young and old cohort, as shown by the statistical similarities of the regressions slopes (Table 5.2).

When normalising nMVC to GM muscle volume (defined as intrinsic strength in the present body of work), there were no associations between the impact of rising adiposity or BMI, and age-specific effects (Table 5.2). However as expected, intrinsic strength in the pooled population was shown to significantly decrease with age ($p<0.001$). This could be explained by denervation of fast motor units (Lang et al. 2010) and a decrease in size of type II fibres (Lexell et al. 1988) associated with ageing. Interestingly at fascicular level when examining the continuum of adiposity against GM skeletal muscle specific force there was no association. However when comparing these findings against the continuum of BMI, GM skeletal muscle specific force in the Y was positively associated with BMI ($r=0.594$; $p=0.042$). This trend is comparable to results reported following 9 weeks of progressive resistance training (Erskine et al. 2010a) and in long term body builders (D'Antona et al. 2006) who have demonstrated an increase in skeletal muscle specific force owing to the continuous loading of the musculature (not dissimilar to long term obesity). The potential mechanism for the rise in specific force may potentially be from an increase in myosin heavy chain isoform content (D'Antona et al. 2006), a higher proportion of fast twitch fibre types observed in obese adults (Kriketos et al. 1997) and/or even preferential Type II fibre

hypertrophy previously observed in obesity induced pig muscle tissue (Clark et al. 2011).

**Impact of BMI and/or adiposity on the magnitude of ageing-related sarcopenia and asthenia**

Ageing related changes to skeletal muscle has been characterised by lower absolute knee extension MVC torque (Goodpaster et al. 2006), decreased muscle activation (Morse et al. 2004), increased co-activation (Klein et al. 2001) and changes in skeletal muscle architecture and size (Morse et al. 2005a). However the effects of obesity on skeletal muscle characteristics on the rate of ageing-related loss of muscle properties had yet to be conducted in male population. The association of age against a series of skeletal muscle variables when categorised by BMI demonstrated that obese male individuals were found to lose nMVC at a similar rate of (i.e. torque decrease per year) as their normal weight counterparts whilst appearing stronger (absolute external torque) and maintaining this advantage throughout life. This appeared to differ when individuals were categorised by total body adiposity, as the high adipose cohort appeared to have higher absolute torque in early years of life, but this strength advantage was not maintained past the fifth decade, in fact demonstrating a functional disadvantage in the high adipose males. Our observations may provide the rationale for the functional difficulties (i.e. kneeling/bending and climbing stairs) reported in elderly obese individuals (Zoico et al. 2004).

The results from this study underpin the detrimental effect of high adiposity and the increased rate of changes observed with ageing through increased muscle content loss (high adipose -1.58$cm^3$/year vs. normal adipose +0.05$cm^3$/years) and GM skeletal muscle specific force (high adipose -0.18 N/$cm^2$ vs. normal adipose -0.02 N/$cm^2$) loss. Interestingly when categorised by BMI, the obese individuals demonstrated negligible losses (-0.15$cm^3$/year) in muscle content associated with ageing (Morse et al. 2005a). This maybe potentially explained by the strong association between BMI and lean mass content in males (Romero-Corral et al. 2008), thus masking the negative effect high adiposity has upon muscle characteristics, particularly with increased age, as further demonstrated in sarcopenic obese individuals (Goodpaster et al. 2006). The potential underpinning mechanism to the acceleration in the loss of both muscle contractile content and skeletal muscle specific force in the presence of high adiposity may be through

high levels of pro-inflammatory cytokines (Cesari et al. 2004; Goodpaster et al. 2006; Roubenoff 1999; Schrager et al. 2007). The acceleration of these two components could exacerbate physical difficulties created through carrying a high amount of inert mass during daily activities (Goodpaster et al. 2001; Rolland et al. 2009; Visser et al. 2005).

In comparison to the present study, chapter 4 examined the rate of ageing on the impact of BMI and/or adiposity on the magnitude of sarcopenia and asthenia in ageing females. Interestingly within this study the only significant effect of combined adiposity and ageing was observed in the rate of loss of GM muscle volume. However, the females within chapter 4 revealed a greater loss in skeletal muscle content from the second to the seventh decade that was -2.1cm$^3$/year in comparison to -1.58cm$^3$/year reported in the males from this study. The potential differences between genders maybe explained due to greater anabolic endocrine profile in males (Baumgartner et al. 1999). However the potential benefit in retaining more muscle in the obese males may not be classified as advantageous, as noted by the increased rate of change observed during ageing in both intrinsic strength at a whole muscle level and specific force at a fascicular level (gender differences will be expanded upon within chapter 6). Further research should focus on the mechanism underpinning the deleterious effects observed in the rate of ageing-related changes, potentially through examining the role of testosterone and oestrogen.

**Conclusion**

This study demonstrates for the first time that the continuum of adiposity and BMI was positively associated with absolute force production and muscle volume in both young and old untrained adult males. Interestingly, the age difference in high adipose individuals was foremost observed through the loss of muscle tissue content, as well supplemented with a greater difference in absolute muscle strength. At the intrinsic level (net torque relative to muscle volume), the age groups difference, as expected, were in fact lesser. Thus, both normal and high adipose males decreased at equal rates. These results differed at a fascicular level, as high adipose individuals were negatively associated with a greater implied decline in GM skeletal muscle specific force with age. These results however need to be explored in greater depth owing to the small sample size in these males participants when compared to the female cohort recruited in

chapters 2-4. However, the potential mechanisms to the implied ageing-related declines in this male cohort may be through high levels of pro-inflammatory cytokines. Indeed cytokines are associated with both ageing and with obesity, a combination highly likely to increase skeletal muscle atrophy. This research highlights the need to therapeutically target high adiposity in the elderly, due to the detrimental effects on skeletal muscle structure and function. This could lead to a decrease in an individual's functional mobility, thus negatively affecting their quality of life (Rolland et al. 2009; Zoico et al. 2004).

# Chapter 6: A between genders contrast of the impact of degree of adiposity on skeletal muscle structural and functional properties changes with ageing

## Abstract

Gender differences in response to the loading of adiposity evident in obesity have been reported in skeletal muscle structure and functional characteristics. However, it remains to be seen whether gender specific differences are transferred to the rate of change associated with ageing, focusing on musculoskeletal variables. This study aimed to investigate the effect of gender when focusing the impact of increasing adiposity vs. age and BMI vs. age on skeletal muscle structural and functional characteristics. Ninety-four untrained females categorised by age into young (Y) (mean ± SD: 25.5 ± 9.0 yrs) versus old (O) (64.8 ± 7.2 yrs) and 34 untrained healthy males categorised by age into young (Y) (mean ± SD: 25.3 ± 9.6 yrs) versus old (O) (68.7 ± 7.2 yrs), were assessed for body composition, gastrocnemius medialis (GM) muscle volume (V), net maximum voluntary contraction (nMVC) correcting for both agonist muscle activation and antagonist co-contraction and GM skeletal muscle specific force (SF). No significant between genders differences were reported in the degree of association (r) or in the slope (Δy:Δx) of regressions when categorised by age, in the relationships between nMVC (Y: z transformation of r 0.50, Student's *t*-statistic 0.072 ; O: z transformation of r 0.55 Student's *t*-statistic 0.075) and V (Y: z transformation of r 0.07, Student's *t*-statistic 0.02 ; O: z transformation of r 0.19, Student's *t*-statistic 0.91) vs. BMI. Interestingly, BMI correlation against GM SF was positive in the Y male cohort (r=0.594; p=0.042) but not the Y female (r=-0.08; p>0.05), therefore the difference between the two slopes was statistically significant (Y male vs. Y female being 0.879 N/cm$^2$/BMI vs. -0.055 N/cm$^2$/BMI; Student's *t*-statistic 3.70, p<0.05). These findings were not seen in the older male and female cohorts (Student's *t*-statistic 0.38, p>0.05 for the continuum of BMI). Remarkably, the rate of deleterious change with increased ageing, implied through regression slopes, in terms of both nMVC and GM V were similar between genders whether obesity was classified using BMI or adiposity. However, the rate of deleterious change with increased ageing, implied through regression slopes, in terms of both nMVC/V (-0.639 Ncm/cm$^3$/year p=0.013) and GM SF (-0.373 N/cm$^2$/year; p=0.007) were significantly steeper in the obese male compared to the obese female. Comparison between genders revealed the slopes to be significantly different (nMVC/V: Student's *t*-statistic -4.09, p<0.05; GM SF: Student's *t*-statistic -4.56, p<0.05)

Obesity (either by high BMI or high adiposity) in the young is associated with loading skeletal muscle in a similar pattern irrespective of gender. However, the

deleterious impact of age vs. GM SF is foremost observed in the obese male. In Fact, obesity accelerates losses of maximum strength and muscle content associated with ageing irrespective of gender. Therefore, this work illustrates why therapeutic interventions into lowering adiposity in later life in both a male and female population are a priority, as these would lead to lessening the risk of mobility limitations.

## Introduction

There remains a scarcity of work detailing whether gender affects skeletal muscle structural and functional characteristics in the obese. However, differences in the functional and structural properties between both young/old (Morse et al. 2005a; Morse et al. 2005b; Rice et al. 1989) and male/female (Frontera et al. 1991; Miller et al. 1993) individuals in a healthy untrained normal weight classification have been extensively examined. The consensus within the literature is that males possess higher absolute strength, which can been attributed to greater absolute muscle mass and larger muscle fibres (Miller et al. 1993). These results have been suggested in an obese population (Lafortuna et al. 2005), yet, the loading effect of adiposity potentially positively affecting skeletal muscle has been reported to differ between genders (Lafortuna et al. 2013).

Lafortuna *et al.* (2005) reported body composition and muscle strength to considerably differ between morbidly obese male and females. Primarily the study demonstrated gender specific differences in body composition. The obese male cohort demonstrated a greater relative fat free mass (estimated through bioelectrical impedance) compared to normal weight male individuals. Whilst the relatively higher fat free mass content of the obese compared to normal weight female, was comparatively lesser than the difference seen in the males, suggesting the loading effect upon the lower limbs of increased BMI would be lower in females. Sartorio *et al.* (2004) supported these findings demonstrating a similar trend in gender specific differences in total fat free mass. In conjunction with these findings, the obese males were shown to possess significantly higher absolute isotonic strength than both their morbidly obese female counterparts and additionally normal weight males. These differences were accounted for through significantly higher fat free mass in both the male and female cohort (Lafortuna et al. 2005).

Interestingly, Lafortuna *et al.* (2013) revealed the loading effect of total body adiposity to differ between genders. This was demonstrated through differences in the slope of regressions between lower limb skeletal muscle volume (measured using computed tomography) against total body adiposity, as the slope of the females was lower in comparison to that of their male counterparts. Both of these studies demonstrate the adaptability of skeletal muscle to the overload created through loading of high levels adipose tissue but also demonstrate the need to systematically investigate gender specific adaptations. This, it is proposed, would be key to addressing potential differences in functional limitations/disability (i.e.

mobility problems) in an obese elderly population in later life (Rolland et al. 2009; Zoico et al. 2004).

When examining the effect gender has upon the rate of ageing of skeletal muscle loss, males have been reported to lose significantly more absolute and relative muscle mass (Janssen et al. 2000). Janssen et al. (2000) reported absolute losses of total skeletal muscle mass to be 3.8kg in females vs. 5.9kg in males from the age 18-88 years old and relative (% skeletal muscle) losses to be 3.9% in females vs. 6.3% in males. However, within this earlier study (Janssen et al. 2000), males were reported to possess a higher amount of skeletal muscle mass than their female counterparts (+40% upper body, +33% lower body), a data set which ties in with previously reported gender differences in muscle strength of both the lower body (+33% males) and upper body (+48% males) (Miller et al. 1993).The apparent male-bias to disadvantage is further supported by data looking at age and gender comparisons in the loss of muscle strength in males and females aged between 20-93 years old (Lindle et al. 1997). However, it remains to be seen whether the age and gender comparisons in the loss of both skeletal muscle mass and strength are continued or even exacerbated in an obese male and female cohort.

The aim of the present study was to investigate if there were gender specific variations in the degree of impact of obesity and/or adiposity on skeletal muscle intrinsic force at both whole muscle and fascicular levels, in both a young and old inactive population. The second aim was to determine whether gender differences were evident in the rate of ageing when classified by either obesity or adiposity status at both whole body and fascicle levels. It was hypothesised that: (1) the loading response of increasing BMI or adiposity on muscle volume would be greater in both Y and O males. (2) The deleterious impact of high BMI or high adiposity on skeletal muscle specific force (at both gross and fascicular levels), would be worse in the male cohort; (3) the rate of ageing (decrease in muscle contractile capacity) would be faster in the presence of a male obese/high adipose profile.

## Methods

**Participants**:

Ninety-four untrained females and thirty-four untrained males volunteered to take part in this study (Table 6.1) and were categorised by age into either Young (Y; 18-49yrs) or Old (O; 50-80yrs). Participants were then sub-categorised into three body mass index classifications (BMI – Body Mass (kg)/Stature$^2$ (m)) into Underweight (BMI <20), Normal (BMI 20-24.9), Overweight (BMI 25-29.9) and Obese (BMI >30.0). Participants were additionally categorised as ordinary-adipose (Females <40%; Males <28%) vs. high-adipose (Females ≥40%; Males ≥28% by body fat percentage) following classification from a previous study (Baumgartner et al. 2004). The exclusion criteria adopted within the study were issues with lower limb muscles/joints affecting mobility or ability to exert maximum strength. Physical activity status was screened by questionnaire and participants were excluded if they self-reported as habitually undertaking structured exercise for more than 3 hours per week and any form of resistance training.

Participants gave written-informed consent prior to undertaking any assessment. All the procedures in this study had approval from the Manchester Metropolitan University Ethics committee and conformed to the standards set by the latest revision (2012-2013) of the Declaration of Helsinki.

**Table 6.1.** Descriptive variables for genders in both young and old age classifications. Data are presented as Mean ± SD.

| **Young (18-49)** | Male (n=16) | Female (n=49) | Gender Effect | Ageing Effect |
|---|---|---|---|---|
| **Age (yrs)** | 25.9±9.5 | 25.5±9.0 | p=0.921 | p<0.001 |
| **Body Mass (kg)** | 82.0±14.7 | 72.1±21.1 | p=0.031 | p=0.710 |
| **BMI (kg/m$^2$)** | 26.1±4.8 | 26.3±7.4 | p=0.513 | p=0.268 |
| **Body Fat %** | 25.1±6.3 | 35.4±8.9 | p<0.001 | p=0.003 |
| **Fat Mass (kg)** | 20.6±8.5 | 26.4±13.7 | p=0.138 | p=0.045 |
| **Lean Mass (kg)** | 55.5±6.7 | 41.5±7.8 | p<0.001 | p=0.050 |
| **Fascicle Pennation Angle (deg)** | 35±2.4 | 31.4±5.4 | p=0.010 | p<0.001 |
| **Fascicle Length (cm)** | 3.5±0.5 | 3.6±0.6 | p=0.568 | p=0.036 |
| **Muscle Volume (cm$^3$)** | 231.1±99.9 | 229.5±82.1 | p=0.308 | p=0.155 |
| **Physiological Cross Sectional Area (cm$^2$)** | 66.3±27.6 | 66.0±22.4 | p=0.286 | p=0.001 |

| **Old (50-80)** | Males (n=18) | Female (n=45) | Gender Effect | Ageing Effect |
|---|---|---|---|---|
| **Age (yrs)** | 68.7±7.2 | 64.8±7.2 | p=0.550 | p<0.001 |
| **Body Mass (kg)** | 83.3±9.6 | 69.4±16.7 | p<0.001 | p=0.710 |
| **BMI (kg/m$^2$)** | 28.0±4.2 | 26.6±5.8 | p=0.294 | p=0.268 |
| **Body Fat %** | 31.0±5.3 | 40.1±6.9 | p<0.001 | p=0.003 |
| **Fat Mass (kg)** | 25.3±6.7 | 27.9±11.0 | p=0.484 | p=0.045 |
| **Lean Mass (kg)** | 52.7±4.7 | 37.4±6.0 | p<0.001 | p=0.050 |
| **Fascicle Pennation Angle (deg)** | 28.1±11.4 | 28.6±4.7 | p=0.277 | p<0.001 |
| **Fascicle Length (cm)** | 3.3±1.4 | 4.0±0.7 | p=0.057 | p=0.036 |
| **Muscle Volume (cm$^3$)** | 235.8±51.7 | 203.1±42.5 | p<0.001 | p=0.155 |
| **Physiological Cross Sectional Area (cm$^2$)** | 56.5±25.2 | 52.0±11.8 | p=0.053 | p=0.001 |

**Protocol order:**

Participants attended the laboratory for testing on two separate occasions. During the first visit, anthropometric measurements (DXA, Stature, Mass) were collected, and the setup and familiarisation with the maximum voluntary contraction (MVC) protocols took place. During the second visit participants gastrocnemius medialis (GM) muscle volume and architecture data were collected prior to conducting the main MVC protocol.

Methodologies for the measurements of body composition measured using DXA, GM moment arm, muscle size and architecture (fascicle pennation angle (FPA)/fascicle length (Lf)) using B-mode ultrasonography and the main MVC protocol (including maximum torque corrected for both agonist muscle activation and antagonist co-activation, classified as net MVC (nMVC)) using an isokinetic dynamometer are extensively detailed in chapters 2-5. Skeletal muscle intrinsic strength at a whole muscle level was defined by nMVC normalised to GM muscle volume and defined as intrinsic strength, whilst at a fascicular level this was classified GM specific force.

**Regression comparison**

Comparison of linear regression coefficients between the continua of BMI, adiposity and age against nMVC, GM muscle volume, intrinsic strength and GM specific force were conducted using the Fisher Z-transformation of r. The Fisher r to z transformation assesses the significance of the difference between two independent linear regression coefficients. Calculation of Z was attained using the following equation:

$$Z = ½ \ln((1+r)/(1-r))$$

Differences between the slopes of the linear regressions were analysed using the Student's *t*-statistic. The student's t-statistic was computed as the difference between the two slopes divided by the standard error of the difference between the slopes. Calculation of Student's *t*-statistic was attained using the following equation:

$$t = (b_1-b_2) / S_{(b_1-b_2)}$$

Where $b_1$ and $b_2$ are the 2 slopes and $S_{(b1-b2)}$ is the standard error of the difference between the two slopes, with

$$S_{(b1-b2)} = \sqrt{((Sb_1)^2 + (Sb_2)^2)}$$

### Statistics

Statistical analyses were carried out using SPSS (Version 19, SPSS Inc., Chicago Illinois). To determine parametricity, Shapiro-Wilk and Levene's tests (homogeneity of variance) were utilised. Pearson and spearman correlation coefficients described the relationships between a continuum of BMI, adiposity and age against nMVC, GM muscle volume, intrinsic strength and GM specific force. Comparison of the regression coefficients and slopes between genders were conducted using z-transformations and the Student's *t*-statistic (Calculations are illustrated above). Significance was reached if $Z > 1.96$, $p<0.05$; or $Z > 2.58$, $p<0.01$. Statistical difference was reported between the slopes of the regressions if the Student's *t*-statistic fell outside ± 1.96 ($p<0.05$).

## Results

### Body composition

Table 6.1 displays the study population characteristics, characterised by both age and gender. As expected, there were significant differences between genders from the pooled categorised age groups for lean mass ($p=0.05$), skeletal muscle FPA ($p<0.001$) and GM physiological cross sectional area ($p=0.001$). Y and O Males had 25% ($p<0.001$) and 29% ($p<0.001$) more lean mass than the Y and O female cohorts respectively. Y males had 10% ($p=0.01$) greater FPA than their Y female counterparts, whilst no differences were reported between the O males vs. O females ($p=0.227$).

**Skeletal Muscle Characteristics**

*BMI and muscle contractile characteristics*

Table 6.2 compared the associations and slopes of the regressions between BMI vs. nMVC, GM muscle volume, intrinsic strength and GM specific force in both Y and O, male and females. The Y male and females had similar associations between BMI vs. nMVC, yet the slope of the regressions indirectly implied the male cohort to increase nMVC at a greater rate with rising BMI (Y male 4.021Nm/kg/$m^2$ vs. Y female 3.233Nm/kg/$m^2$) (Figure 6.1.A). However, the differences between these slopes were not statistically significant (Y Student's *t* statistic 0.72; $p>0.05$). This trend between associations and slopes of regressions were repeated in the older cohort against BMI vs. nMVC (Table 6.2).

In the Y cohort, the degree of association in the BMI vs. GM muscle volume relationships were similar between the two genders. However, the slopes of these regressions implied that both the males and females increased muscle content at a similar rate with rising BMI (Figure 6.1.B). In the older cohort, the degree of association in the BMI vs. GM muscle volume relationships were also similar between the two genders. However, the slope of the regression implied the O male cohort to increase muscle volume at a greater rate similar to the results examining the BMI vs. nMVC relationship in the old.

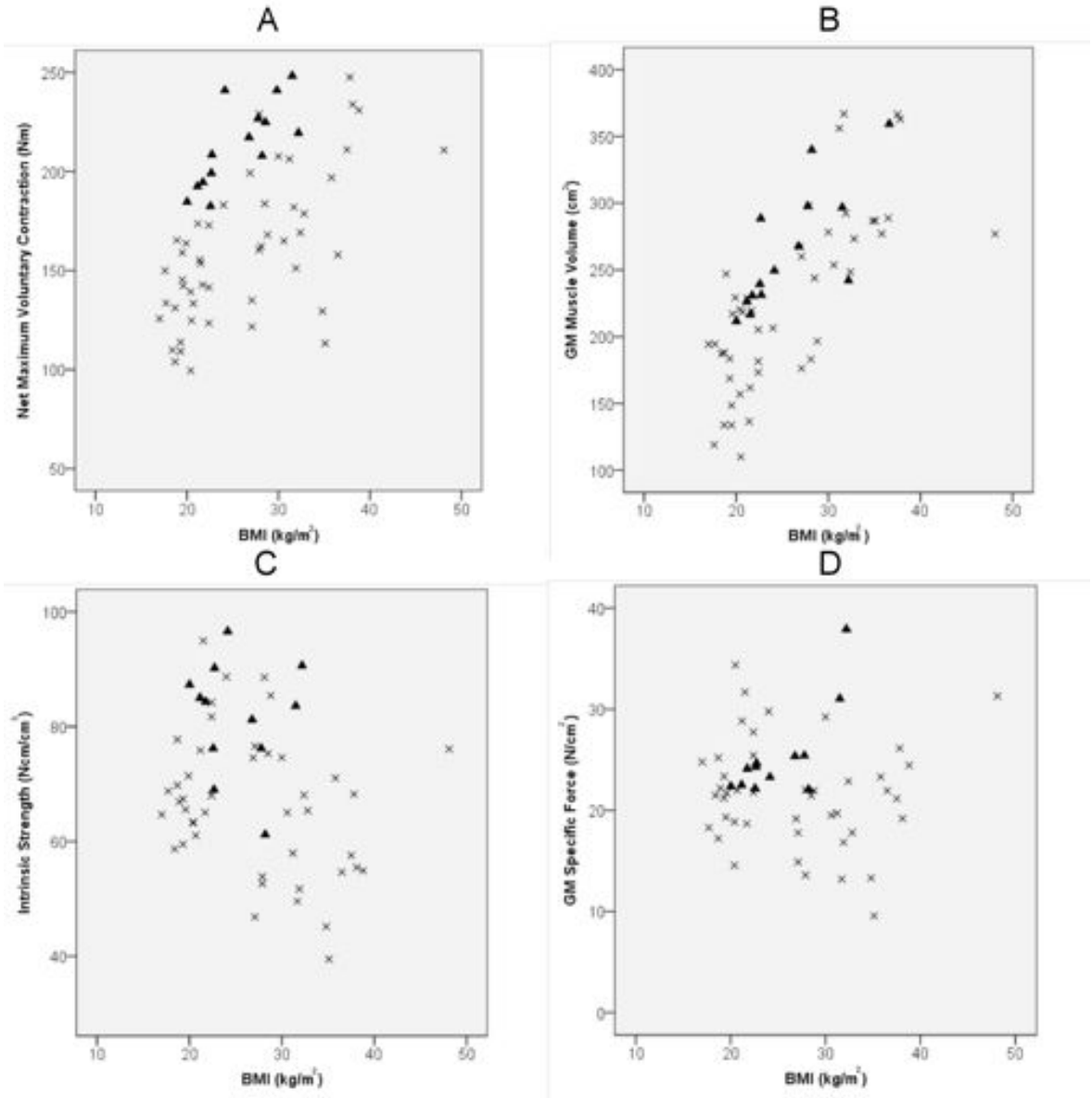
There were no differences reported in the degree of association or slopes of the regressions between the BMI vs. intrinsic strength relationship in both Y and O, males and females.

At a fascicular level, both the degree of association and slopes of the regressions were statistically different in the Y cohort (Table 6.2). The slopes of the regressions implied the Y males to increase GM specific force with rising BMI, in comparison to the Y females losing specific force with increasing BMI (Y male 0.879 N/$cm^2$/kg/$m^2$ vs. Y female -0.055 N/$cm^2$/kg/$m^2$). However, there were no differences reported in either the associations or slopes of the regressions in the older cohort.

**Table 6.2.** Pearson correlations, z-transformation of r and Student's *t*-statistic between net maximum voluntary contraction (nMVC), gastrocnemius medialis (GM) muscle volume, intrinsic strength and GM specific force against a continuum of BMI in young and old untrained male and females (* P<0.05, ** P<0.01, *** P<0.001) (If Z > 1.96, p<0.05; Z > 2.58, p<0.01) (Student's *t*-statistic significance if *t* falls outside ± 1.96 p<0.05)

| | Male | | | Female | | Correlation co-efficient | Gender Effect | |
|---|---|---|---|---|---|---|---|---|
| | n | r value | slope | n | r value | slope | Z-transformation of r | Student's *t*-statistic |
| **Young** | | | | | | | | |
| **nMVC vs. BMI** | 14 | 0.76** | 4.02 Nm/kg/m$^2$ | 49 | 0.65*** | 3.23 Nm/kg/m$^2$ | 0.50 | 0.72 |
| **Muscle Volume vs. BMI** | 14 | 0.76** | 7.04 cm$^3$/kg/m$^2$ | 46 | 0.75*** | 6.92 cm$^3$/kg/m$^2$ | 0.07 | 0.02 |
| **Intrinsic Strength vs. BMI** | 12 | 0.10 | -0.24 Ncm/cm$^3$/kg/m$^2$ | 44 | 0.28 | -0.47 Ncm/cm$^3$/kg/m$^2$ | -0.85 | 0.28 |
| **GM Specific Force vs. BMI** | 12 | 0.59* | 0.88 N/cm$^2$/kg/m$^2$ | 44 | 0.08 | -0.05 N/cm$^2$/kg/m$^2$ | 2.11* | 3.70* |
| **Old** | | | | | | | | |
| **nMVC vs. BMI** | 18 | 0.54* | 3.08 Nm/kg/m$^2$ | 45 | 0.41** | 1.95 Nm/kg/m$^2$ | 0.55 | 0.75 |
| **Muscle Volume vs. BMI** | 18 | 0.47* | 5.75 cm$^3$/kg/m$^2$ | 45 | 0.43** | 3.13 cm$^3$/kg/m$^2$ | 0.19 | 0.91 |
| **Intrinsic Strength vs. BMI** | 17 | 0.04 | -0.08 Ncm/cm$^3$/kg/m$^2$ | 43 | 0.06 | 0.11 Ncm/cm$^3$/kg/m$^2$ | -0.21 | -0.30 |
| **GM Specific Force vs. BMI** | 15 | 0.14 | 0.14 N/cm$^2$/kg/m$^2$ | 44 | 0.03 | 0.03 N/cm$^2$/kg/m$^2$ | 0.93 | 0.38 |

**Figure 6.1.** Displays linear regressions on the impact of BMI on net maximum voluntary contraction (A), gastrocnemius medialis (GM) muscle volume (B), GM intrinsic strength (C) and GM specific force (D) in the young, categorised by gender. (Male =▲ ; Female = ×)




*Adiposity and muscle contractile characteristics*

Table 6.3 compared the associations and slopes of regressions between total body adiposity vs. nMVC, GM muscle volume, intrinsic strength and GM specific force in both the Y and O, males and females. The Y males and females had similar associations between total adiposity vs. nMVC, yet the slope of the regressions implied the female cohort to increase nMVC at a greater rate with rising total body adiposity (Y male 1.561Nm/kg vs. Y female 1.654Nm/kg).

However, the differences between these slopes were not statistically significant (Y Student's *t*-statistic -0.12; p>0.05). The O male and females had similar associations between total adiposity vs. nMVC, yet the slope of the regressions implied the male cohort to increase nMVC at a greater rate with rising total adiposity (O male 1.605Nm/kg vs. O female 0.948Nm/kg). However, the differences between these slopes were not statistically significant (O Student's *t*-statistic 0.67; p>0.05).

In the Y cohort, the degree of association in the total body adiposity vs. GM muscle volume relationships were similar between the two genders, yet the slope of the regressions implied the female cohort to increase GM muscle volume at a greater rate with rising total body adiposity (Y male 3.641$cm^3$/kg vs. Y female 4.292$cm^3$/kg). However, the differences between these slopes were not statistically significant (Y Student's *t*-statistic -0.56; p>0.05). The O male and females had similar associations between total adiposity vs. nMVC, yet the slope of the regressions implied the male cohort to increase GM muscle volume at a greater rate with rising total adiposity (O male 4.222$cm^3$/kg vs. O female 1.523$cm^3$/kg). However, the differences between these slopes were not statistically significant (O Student's *t*-statistic 1.59; p>0.05).

There were no differences in the degree of association or slopes of the regressions between the total body adiposity vs. intrinsic strength relationship in both Y and O, male and females.

At a fascicular level, the Y male and females had similar associations between the total body adiposity vs. GM specific force relationship, yet the slope of the regressions implied the male cohort to increase GM specific force at a greater rate with rising total body adiposity, in comparison to the Y females losing specific force with increasing total body adiposity (Y male 0.408N/$cm^2$/kg vs. Y female -0.113N/$cm^2$/kg). The differences between these slopes were statistically significant (Y Student's *t*-statistic 3.17; p<0.05). These findings were not reiterated in the older cohort, as no differences were revealed between both the strength of associations and slopes of the regressions.

**Table 6.3.** Pearson correlations, z-transformation of r and Student's *t*-statistic between net maximum voluntary contraction (nMVC), gastrocnemius medialis (GM) muscle volume, intrinsic strength and GM specific force against a continuum of adiposity in young and old untrained male and females (* P<0.05, ** P<0.01, *** P<0.001) (If Z > 1.96, p<0.05; Z > 2.58, p<0.01) (Student's *t*-statistic significance if *t* falls outside ± 1.96 p<0.05)

| | Male | | | Female | | | Correlation co-efficient | Gender Effect |
|---|---|---|---|---|---|---|---|---|
| | n | r value | slope | n | r value | slope | Z-transformation of r | Student's *t*-statistic |
| **Young** | | | | | | | | |
| **nMVC vs. Adiposity** | 14 | 0.52 | 1.56 Nm/kg | 48 | 0.64*** | 1.65 Nm/kg | -0.46 | -0.12 |
| **Muscle Volume vs. Adiposity** | 14 | 0.71** | 3.64 $cm^3$/kg | 47 | 0.80*** | 4.29 $cm^3$/kg | -0.45 | -0.56 |
| **Intrinsic Strength vs. Adiposity** | 12 | 0.17 | -0.23 Ncm/$cm^3$/kg | 44 | 0.35* | -0.31 Ncm/$cm^3$/kg | -0.73 | 0.20 |
| **GM Specific Force vs. Adiposity** | 12 | 0.31 | 0.41 N/$cm^2$/kg | 48 | 0.23 | -0.11 N/$cm^2$/kg | 0.30 | 3.17* |
| **Old** | | | | | | | | |
| **nMVC vs. Total Adiposity** | 18 | 0.59* | 1.61 Nm/kg | 45 | 0.38* | 0.95 Nm/kg | 0.89 | 0.67 |
| **Muscle Volume vs. Adiposity** | 18 | 0.55* | 4.22 $cm^3$/kg | 45 | 0.40* | 1.52 $cm^3$/kg | 0.65 | 1.59 |
| **Intrinsic Strength vs. Adiposity** | 17 | 0.23 | -0.30 Ncm/$cm^3$/kg | 45 | 0.02 | 0.02 Ncm/$cm^3$/kg | 1.54 | -0.88 |
| **GM Specific Force vs. Adiposity** | 15 | 0.06 | -0.04 N/$cm^2$/kg | 45 | 0.11 | 0.05 N/$cm^2$/kg | -0.34 | -0.28 |

*Degree of association between age and muscle size and/or strength by obesity status*

Table 6.4 compared the associations and slopes of regressions between age vs. nMVC, GM muscle volume, intrinsic strength and GM specific force in both the obese and high adipose, males and females. Starting with the genders classified obese by their BMI, the obese male and females had similar associations between age vs. nMVC, yet the slope of the regressions implied the male cohort to lose nMVC at a greater rate with rising age (obese male -1.250Nm/year vs. obese female -1.150Nm/year). However, the differences between these slopes were not statistically significant (Student's *t*-statistic -0.11; p>0.05).

The degree of association in the age vs. GM muscle volume relationships were similar between the two genders, yet the slope of the regressions implied the obese female cohort to lose GM muscle volume at a greater rate with rising age (obese male -0.148cm$^3$/kg vs. obese female -2.104cm$^3$/kg). However, the differences between these slopes were not statistically significant (Student's *t*-statistic 1.58; p>0.05).

The obese males and females had similar associations between the age vs. intrinsic strength relationship, yet the slope of the regressions implied the male cohort to lose intrinsic strength at a greater rate with rising age, in comparison to the obese females increasing intrinsic strength with increasing age (obese male (-0.639Ncm/cm$^3$/year vs. obese female 0.085Ncm/cm$^3$/year). Here, the differences between these slopes were statistically significant (Student's *t*-statistic -4.09; p<0.05)

At the fascicular level, the obese males and females had similar associations between the age vs. GM specific force relationship, yet the slope of the regressions implied the male cohort to decrease GM specific force at a greater rate with rising age, in comparison to the obese females losing specific force with increasing age (obese male -0.373N/cm$^2$/year vs. obese female 0.056N/cm$^2$/year). The differences between these slopes were also in fact statistically significant (Student's *t*-statistic -4.56; p<0.05).

Moving onto genders classified high adipose by body fat percentage, the high adipose males and females had similar associations between age vs. nMVC, yet the slope of the regressions implied the male cohort to lose nMVC at a greater rate with rising age (high adipose male -1.576Nm/year vs. high adipose female -1.259Nm/year). However, the differences between these slopes were not statistically significant (Student's *t*-statistic -0.85; p>0.05).

The degree of association in the age vs. GM muscle volume relationships were similar between the two genders, yet the slope of the regressions implied the high adipose female cohort to lose GM muscle volume at a greater rate with rising age (high adipose male -1.577cm$^3$/kg vs. obese female -2.104cm$^3$/kg). However, the differences between these slopes were not statistically significant (Student's *t*-statistic 0.68; p>0.05).

The ageing vs. intrinsic strength loss relationship revealed the degree of association to be statistically different between genders (Table 6.4). The slope of the regressions implied the male cohort to lose intrinsic strength at a greater rate with rising age (high adipose male -0.310 Ncm/cm$^3$/year vs. high adipose female

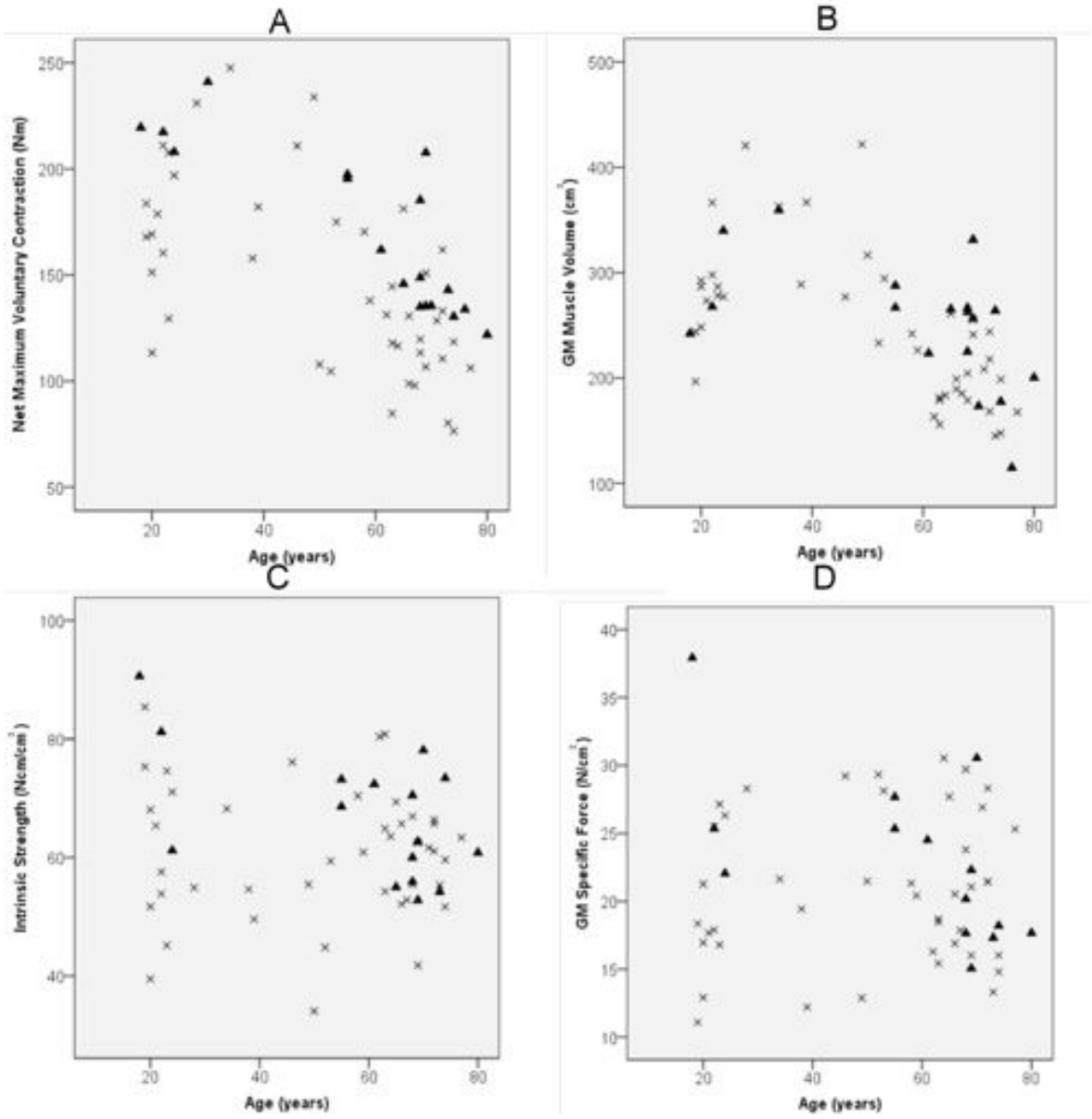
0.018 Ncm/cm$^3$/year). However, the differences between these slopes were not statistically significant (Student's *t*-statistic -1.95; p>0.05).

Finally at the fascicular level, both the degree of association and slopes of the regressions were statistically different in the high adipose cohort (Table 6.4 & Figure 6.2.D). The slopes of the regressions implied the Y males to decrease GM specific force with rising age, in comparison to the high adipose females increasing specific force with increasing age (high adipose male (-0.183 N/cm$^2$/year vs. high adipose female 0.12 N/cm$^2$/year).

**Table 6.4.** Pearson correlations, z-transformation of r and Student's *t*-statistic between net maximum voluntary contraction (nMVC), gastrocnemius medialis (GM) muscle volume, intrinsic strength and GM specific force against age in untrained male and females classified obese by either BMI or adiposity. (* P<0.05, ** P<0.01, *** P<0.001) (If Z > 1.96, p<0.05; Z > 2.58, p<0.01) (Student's *t*-statistic significance if *t* falls outside ± 1.96 p<0.05)

| | Male | | | Female | | Correlation co-efficient | Gender Effect | |
|---|---|---|---|---|---|---|---|---|
| | n | r value | slope | n | r value | slope | Z-transformation of r | Student's *t*-statistic |
| **BMI (Obese)** | | | | | | | | |
| **nMVC vs. Age** | 6 | 0.60 | -1.25 Nm/year | 27 | 0.50** | -1.15 Nm/year | 0.21 | -0.11 |
| **Muscle Volume vs. Age** | 7 | 0.06 | -0.15 cm$^3$/year | 25 | 0.70*** | -2.10 cm$^3$/year | -1.79 | 1.58 |
| **Intrinsic Strength vs. Age** | 6 | 0.91* | -0.64 Ncm/cm$^3$/year | 26 | 0.18 | 0.09 Ncm/cm$^3$/year | 1.86 | -4.09* |
| **GM Specific Force vs. Age** | 6 | 0.93** | -0.37 N/cm$^2$/year | 27 | 0.19 | 0.06 N/cm$^2$/year | 1.95 | -4.56* |
| **Adiposity (High Adipose)** | | | | | | | | |
| **nMVC vs. Age** | 18 | 0.87*** | -1.58 Nm/year | 43 | 0.60*** | -1.26 Nm/year | 1.51 | -0.85 |
| **Muscle Volume vs. Age** | 18 | 0.52* | -1.58 cm$^3$/year | 43 | 0.65*** | -2.10 cm$^3$/year | -0.59 | 0.68 |
| **Intrinsic Strength vs. Age** | 16 | 0.56* | -0.31 Ncm/cm$^3$/year | 43 | 0.03 | -0.02 Ncm/cm$^3$/year | 2.69* | -1.95 |
| **GM Specific Force vs. Age** | 14 | 0.62* | -0.18 N/cm$^2$/year | 43 | 0.12 | 0.03 N/cm$^2$/year | 2.07* | -2.71* |

**Figure 6.2.** Displays linear regressions on the impact of age on adiposity classification in male and females on net maximum voluntary contraction (A), gastrocnemius medialis (GM) muscle volume (B), intrinsic strength (C) and GM specific force (D).
(High Adipose Male =▲ ; High Adipose Female = ×)




## Discussion

This study was the first to demonstrate gender differences in the rate of ageing of obese and high adipose individuals at the fascicular level (specific force). In regression analyses when BMI was used as the independent variable against nMVC, the slope of the regressions implied both the young and old males

to increase torque at a greater rate than the female young/old cohort. In conjunction with the BMI vs. nMVC relationship, both the slopes of the regressions from the BMI vs. GM muscle volume relationship implied the Y and O male cohorts to increase GM muscle volume at slightly greater rate with increasing BMI, supporting our first hypothesis. At a fascicular level the effect of increasing BMI elicited different effects between genders, as demonstrated by significant differences between the slopes of the regressions. This effect was most pertinent in the younger cohort, as increasing BMI had a positive effect on the slope of regression rejecting our 2$^{nd}$ hypothesis, in comparison to the female cohort, whose slope decreased with rising BMI. The overall trend was repeated when adiposity was utilised as a variable instead of BMI.

The rate of ageing in terms of nMVC loss was found to be worse in the male obese cohort whether classified by BMI or adiposity, partially supporting our final hypotheses. However, contrary to our final hypothesis, muscle volume tended to decrease at a greater rate in the obese/high adipose female cohort, yet these trends for differential slopes were not significant between genders. When classified obese using BMI, differences were reported between slopes in the rate of change of intrinsic strength loss with ageing. The slopes of the linear regressions implied the obese male cohort to significantly lose intrinsic strength at a greater rate per year in comparison to the female cohort. Finally, gender differences were reported between the slopes of regressions whether classified as either obese or high adipose in the GM specific force ageing relationship. Unexpectedly, only the obese/high adipose male cohort slopes of the regressions implied them to lose specific force with increasing age in comparison to the female cohort. Therefore, based on these slopes, it can be indirectly implied that whether classified obese by either BMI or adiposity the obese males were associated with faster detrimental fascicular changes, than their obese/high adipose female counterparts.

**Gender differences from both the continuum of adiposity or BMI on skeletal muscle characteristics**

It is evident that increasing BMI and adiposity acts as a loading stimulus to skeletal muscle structure and function in both a male and female population (as demonstrated in chapters 4 and 5). However, assessment of the literature on gender-related variations on the skeletal response to obesity is scarce. Lafortuna

et al. (2005) reported maximal isotonic strength to increase at similar rates between young obese men and women. This is confirmed within the current study, as no statistical differences were reported between associations and slopes of the regressions in both young and old individuals, when against either a continuum of BMI (Table 6.2) or adiposity (Table 6.3). The findings from the regression slopes implied that when using BMI as a continuum, the male individuals to be apparently stronger with nMVC increasing at a slightly greater rate. This finding however appeared not to be mirrored when exchanging BMI for adiposity in the Y individuals, in regard to the steepness of the slopes.

In conjunction with these findings, the loading response on GM muscle volume of either rising BMI or adiposity elicited a similar response of that witnessed in the linear regressions when maximal strength was the dependent variable. This finding is partially supported within the literature as previous studies have demonstrated a positive association between rising BMI or adiposity and muscle mass in both genders (Lafortuna et al. 2005; Lafortuna et al. 2013; Sartorio et al. 2004). Yet, all three studies report a greater positive effect upon total fat free mass (Lafortuna et al. 2005; Sartorio et al. 2004) and lower limb muscle volume (Lafortuna et al. 2013) in male individuals with rising BMI. This study supports this theorem against a continuum of BMI, yet not when associations utilise total body adiposity. As expected the linear regressions implied the male individuals to have apparently greater muscle volume. Yet, the slopes of the regressions scaled using BMI, implied the male cohort to increase both GM muscle volume at a slightly greater rate supporting one of the hypotheses. However, out of the three studies mentioned, Lafortuna et al. (2013) quantified muscle volume of the entire lower limb using computed tomography. Nevertheless, a limitation of this study potentially effecting the interpretation of the results was due to the relatively small sample size (21 men vs. 18 women) coupled with a large age range (31-71 years old). The ramification of quite a large age range used within this study (Lafortuna et al. 2013) is the potential to falsely lower the slope of the regression due to the blunted response in increasing muscle volume witnessed in elderly obese females (as demonstrated in chapter 3). Therefore, comparison into the effect of obesity between genders should be kept within specific age classifications to avoid potential erroneous conclusions.

Interestingly at a fascicle level, comparison of the associations and slopes of the regressions revealed significant differences between the young males and females. The slopes of the regressions implied the male cohort to increase GM

specific force with increasing BMI, whilst the Y female cohort decreased GM specific force with increasing BMI. The increase in GM specific force in the Y male cohort is similar to what is witnessed following progressive resistance training (Erskine et al. 2010b). Therefore, if using resistance training as an example for the increase in specific force, it is hypothesised that the increase in GM specific force with increased BMI, maybe a result of an increase in myosin heavy chain composition as observed in male bodybuilders (D'Antona et al. 2006). Interestingly, these results presented in this chapter were not matched in an elderly cohort, potentially owing to the generally observed denervation of type II fibres associated with ageing (Larsson et al. 1979; Lexell et al. 1983), as well as reduced levels of hypertrophic hormones (Rudman et al. 1981) which would lower the response obesity elicits on skeletal muscle in older age. However, as the present body of work is the only study to examine gender differences at the fascicular level in an obese cohort, future research should aim to increase sample size of the male cohort to confirm these preliminary findings. In conjunction with increasing the sample size, muscle biopsies would aide in confirming hypotheses generated from this study regarding obesity increasing both muscle fibre size and myosin heavy chain content.

**Gender variation in the degree of association between age and muscle size and/or strength by BMI and adiposity status**

The effects of obesity whether classified by BMI or adiposity have been shown to increase the deleterious effects of ageing in relation to both maximum torque loss and muscle content loss in both a male (chapter 5) and female (chapter 4) population. When comparing associations and slopes of regression between genders there were no statistical differences revealed between associations implying the disadvantage of obesity is similar between genders. However, the slopes of the regressions implied the obese male cohort to lose maximum strength at a greater rate than the obese female cohort. Yet, when nMVC loss was exchanged for muscle content loss, the slopes of the regressions implied the female cohort whether classified obese by BMI or adiposity to lose GM muscle volume at a greater rate (Table 6.4). Whilst both of these differences were not significant, the trend of an increased loss of maximum torque associated with increasing age in males is similar to previously reported work (Clement 1974; Hurley 1995; Lindle et al. 1997). However, the current study conflicts with previous

literature who reported muscle content to decrease at greater rate in males (Janssen et al. 2000). When critiquing these findings against the current study, the sample used by Janssen *et al.* (2000) did not segregate either the male and female population by obesity classification. However, in order to confirm the findings from this study the sample size needs to be increased to similar levels demonstrated in the work produced by Janssen *et al*. (2000) (34 vs. 268 men).

Finally, when exploring gender variations on the effect of age related changes at both a gross or fascicular level, the slopes of the regressions revealed significant differences between genders. When classified obese by BMI, both intrinsic strength and GM specific force revealed significant differences from the slopes of regressions between genders. As expected within the literature (Jubrias et al. 1997; Morse et al. 2005b; Reeves et al. 2004b) the male obese cohort decreased force at both a gross and fascicular level, as implied through the slope of the regressions. Yet, whilst there was no association between intrinsic strength and GM specific force in the obese female cohort, the slopes of the ageing regressions implied the obese females to maintain force at both a gross and fascicle throughout life (i.e. from young to older age). These reports were partially confirmed when adiposity was used in the classification of obesity in individuals, a trend was followed between both intrinsic strength and GM specific force, yet only significant differences were only reported between genders for slopes of regression of age vs. GM specific force.

## Conclusion

Previous chapters have shown that both BMI and adiposity are associated with an increase in skeletal muscle maximal strength and size in both men and women. However, this study was the first to extensively examine gender differences between muscle characteristics quantified at both a gross (intrinsic strength) and fascicle (specific force) level. Gender comparisons revealed the associations and slopes of regressions to not be statistically different whilst segregated by age classification and using either BMI or adiposity as a continuum against maximum strength, GM muscle volume and intrinsic strength. However, statistical differences arose between genders at a fascicular level, with the continuum of increasing BMI or total body adiposity eliciting a positive effect on the slope of the regressions of GM specific force in the young male cohort, but not so in the young females.

Interestingly, when obesity was classified using BMI in both men and women, the deleterious effects of ageing were foremost observed at both a gross and fascicle level in the male cohort. However, statistical significance was only repeated at a fascicle level when classified obese by adiposity. Interpretation of these results suggests that obesity by definition irrespective of classification method accelerates the ageing related loss of force at a fascicular level in males when compared against females. The explanation may be due preferential loss of type II fibres in the male cohort, therefore decreasing maximum strength capability, yet this has not been previous researched in obese elderly males. Implications of the gender related differences in the ageing response coupled with obesity suggest that lowering adiposity in men, arguably more so than in women, is imperative to help maintain functional capability in later life, thus lowering the risk of increased falls (Campbell et al. 1989) and hip fractures (Aniansson et al. 1984) associated with muscle weakness. However, this conclusion would need to be confirmed through increasing the sample size of the young and old males to gain a more reliable description of the interrelation between ageing and obesity in males, and thence, any gender differences in the incidence of rate of deleterious changes to muscle structure and/or function in adult populations of differing body compositions.

# Chapter 7: General Discussion & recommendations for future research

## General Discussion

The aim of this thesis was to examine the impact of body composition on skeletal muscle structural and functional properties, with the emphasis being on obesity and its impact in young and old untrained populations.

The review of the current literature (chapter 1), highlighted to the reader the physiological and biomechanical determinants of skeletal muscle strength and presented the current status of knowledge about skeletal muscle strength in obese adolescent, young adult as well as old adult populations, to identify gaps within the current literature. Information gathered from said literature led to hypotheses including that the extra inert mass carried by an obese individual would act as a loading stimulus to anti-gravity musculature of the lower limbs, similar to strength gains witnessed from hypergravity training (Bosco 1985; Bosco et al. 1986; Bosco et al. 1984). However, the average duration of the stimulus of high adiposity when classified obese (Abdullah et al. 2011), is dissimilar to the acute period the individuals undertaking hypergravity training were placed in (average 13 years vs. 3 weeks). Therefore, whilst the adaptation response during hypergravity training is hypothesised to being neural in origin (i.e. from increased fire frequency and motor recruitment of the muscle), it was proposed that adaptations might be both myogenic and neural due the average duration that an individual spends being obese (Abdullah et al. 2011). Based on this hypothesis, the question arose as to what extent obesity/high adiposity elicits changes on skeletal muscle structure and function. Specifically, would the presence of obesity change the phenotype for variables such as agonist muscle activation, antagonist co-contraction, muscle physiological cross sectional area (PCSA), muscle architecture and moment arm length, in young and old male and female obese populations?

The starting point (Chapter 2) of the data section of the thesis was to investigate the neuromuscular determinants of muscle strength, namely agonist muscle activation and antagonist co-activation in an obese young (18-49) and old (50-80) female population. Obesity within this chapter was classified using both an individual's body mass index (BMI) and body fat percentage (>40%) (Baumgartner et al. 1999). The distinction was made due to the inherent limitation of BMI not being able to distinguish between bone, lean and fat tissue within the calculation. In addition, whilst loading of the lower limbs would occur, all loads modality may not be equal in that the extra load of adiposity per se would mask any overall loading benefits, an effect assumed to be linked to the presence of cytokines such

as Tumour Necrosis Factor α (TNF-α) and interlukin-6 (IL-6) in particular (Visser et al. 2002). Calculation of plantar flexor (PF) maximum voluntary contraction (MVC) was recorded and later corrected for antagonist co-contraction and agonist muscle activation (classified nMVC) (Erskine et al. 2009). Previous studies (Abdelmoula et al. 2012; Blimkie et al. 1990; Hilton et al. 2008; Hulens et al. 2002; Hulens et al. 2001; Rolland et al. 2004; Zoico et al. 2004) had only reported raw MVC without correction for these neuromuscular determinants of muscle strength, potentially leading to an under/overestimation in the true maximum torque potential of an obese individual. Interestingly, muscle activation was shown not to be significantly lower in the old female population irrespective of BMI classification (p=0.138). However, when obesity was classified using adiposity, the young obese/high adipose females reported significantly lower agonist muscle activation (88 vs. 94 %; p=0.019) but the older obese/high adipose females (83 vs. 87%; p=0.458) did not. These findings demonstrated that young high adipose females are at a significant disadvantage due to the inability to recruit their maximum strength capability. In addition, regardless of BMI/obesity status, there was an obligatory decrease in activation capacity comparing young against older females. This disadvantage will be foremost observed in body weight tasks that require strength. The suggested mechanism of lower muscle activation capability especially in the old is often linked to lower habitual physical activity levels (Morse et al. 2004). This hypothesis can also be suggested for a young obese cohort, as previous research has shown both obese adult male and females to exhibit higher degrees of sedentarism (Tjepkema 2006). However, within the current thesis, the physical activity status of participants was screened by questionnaire and participants were excluded if they self-reported as habitually undertaking structured exercise for more than 3 hours per week and any form of resistance training. In addition, the elderly cohort recruited for this study were community dwelling and independently living individuals who were excluded if they presented with any issues with lower limb muscles/joints affecting mobility or ability to exert maximal torque (e.g. Osteoarthritis (Alnahdi et al. 2012)).

Therefore, due to the screening of physical activity levels in participants, it is suggested here that the mechanism involved in lowering muscle activation in the young may potentially be associated with high levels of adiposity, specifically intra muscular adipose tissue (IMAT). Interestingly, IMAT is not only associated with obesity (Hilton et al. 2008) but old age as well (Delmonico et al. 2009). This may potentially explain why there were no between group differences in the in the

elderly cohort irrespective of obesity classification. In other words, there may be an adiposity threshold beyond which further impairment in muscle activation capacity is not seen. IMAT is associated with metabolic impairments within skeletal muscle, such as insulin resistance and risk of developing diabetes mellitus (type II) (Hu et al. 2001; Goodpaster et al. 2000). The metabolic impairment created is linked to peripheral neuropathy, a nervous system disease that is characterised by motor dysfunction and muscle weakness (Andersen et al. 1996). The present body of PhD research somewhat controlled for the possibility of subjects having diabetes mellitus (and thus peripheral neuropathy) by taking fasted blood glucose samples. However, the collection of one-off fasted blood samples does not categorise metabolic impairments at the muscle level, it only indicates the possibility of presence of diabetes mellitus. A more stringent and accurate measure in the diagnosis of diabetes would be an insulin clamp test quantifying both insulin secretion and resistance of an individual. Future research, should examine the pathways and mechanisms involved in the neural impairment evident in a young obese population. Final analysis of these findings would tend to refute the suggestion by Bosco *et al.* (1986) of a neurogenic mechanism for increased muscle power as a result of hypergravity training, being attributed to a similar response in the obese. Therefore, it is proposed instead that any strengthening effect of obesity would be through muscular factors such as increased protein synthesis rate (Burd et al. 2010), which is evidenced through the phenotypic expression of greater absolute lean mass in this population. The muscular events were the topic of the next chapter.

Following on from the effect of body composition on neuromuscular determinants of skeletal muscle strength, chapter 3 focused on the morphological determinants of maximum strength, chronicling the effect of body mass, BMI and adiposity against gastrocnemius medialis (GM) muscle architecture and size. To the author's knowledge, this study was the first to compare the effects of body composition on skeletal muscle fascicle pennation angle (FPA) and fascicle length (Lf). As expected, FPA was shown to decrease with age, confirming previous findings into the ageing response of skeletal muscle (Narici et al. 2003). However, for the first time FPA was shown to increase with BMI classification at both rest and during MVC in both young (rest 15%, 23% and 1%; max 25%, 25% and 13%) and old (rest 38%, 20% and 8%; max 32%, 22% and 10%) untrained females (for underweight, normal, overweight people, respectively). it was proposed that the increase in FPA may partially explain the increase observed in MVC torque in both

the young and older obese cohorts detailed in the previous chapter (i.e. chapter 2). Indeed, an increase in FPA allows for more sarcomeres to be arranged in parallel, which suggests hypertrophy at the single fibre level (Clark et al. 2011). However, the increase in FPA is only beneficial as long as the increase in FPA does not exceed 45° at which point the resultant force resolved at the tendon becomes negative due to the principles of trigonometry (Alexander and Vernon 1975; Degens et al. 2009). These findings suggested that adiposity loads skeletal muscle in a similar manner to progressive resistance training (Erskine et al. 2010b), in particular male bodybuilders who load their muscles on a daily basis (D'Antona et al. 2006). Interestingly, Lf was not observed to change between either BMI ($p=0.376$) or age ($p=0.063$) classifications suggesting that obesity increases sarcomeres in parallel, yet not in series. The functional consequence of a shortened Lf is a decrease in the number of sarcomeres in series, with a potential twofold effect: (a) an alteration to the working range of the muscle, where this unit may adapt by exhibiting a change in its force-length relationship, shifting to a shorter muscle length for peak force; (b) a decrease in the muscle shortening velocity, and ultimately the muscle maximum power generation capacity. This cascade of effects would potentially cause problems for an obese or elderly population in activities such as locomotion and tasks involving the need to apply forces at relatively high velocities (such as, in getting up from a chair to answer a doorbell ring for instance). However it is important to note, that the impact of obesity on tendon properties have yet to be investigated, thus our conclusions on Lf must be tempered. Indeed a more compliant tendon with obesity could give the impression of a shorter Lf, ruling out the suggestion of a loss of sarcomeres in series.

Whilst the increase in maximal torque (in the young obese) can partially be attributed to architectural changes, a further contributing factor may be the increase in PCSA observed in the young female cohort. In fact, PCSA was shown to significantly increase in the young obese cohort when compared against their young underweight (+77%; $p<0.001$), normal weight (+70%; $p<0.001$) and overweight (+31%; $p=0.017$) counterparts, whilst no between group differences were reported in the older female cohort. The lack of between group differences in PCSA in the older population was aligned with a lower GM muscle volume evident in all BMI classifications. The rationale inferred through previous literature in chapter 3 in regards to the disparate response observed between the young and old obese females, was through higher levels of circulating pro-inflammatory

cytokines seen in both obese and sarcopenic obese individuals (Schrager et al. 2007; Hotamisligil et al. 1995). Cytokines that were identified as being of particular interest were IL-6 and TNF-α, both of which have been shown to negatively correlate with muscle strength and muscle mass in the elderly (Visser et al. 2002). The production of high levels of such pro-inflammatory cytokines is reported to increase catabolic activity within skeletal muscle (Roubenoff et al. 1997). Accompanying the catabolic environment, is the reduction in anabolic signalling of growth hormones such as insulin like growth factor-1 (IGF-I), which has been reported in both elderly (Bucci et al. 2013) and severely obese male and females (Williams et al. 1984). Therefore, the potential synergistic action of increased catabolism and decreased anabolism may explain 'combined ageing and obesity'-induced losses in GM muscle tissue content. The most comprehensive previous study to have examined the continuum of total body adiposity and its impact on skeletal muscle volume in the lower limbs, used computed tomography (Lafortuna et al. 2013). However, within their study, the authors did not segregate by age, and in fact utilised a large age range spanning from 32 to 76 years. This study (Lafortuna et al. 2013) reported the response to loading from adiposity differed between genders, yet taking into account the results from Chapter 3 in the current thesis, it was demonstrated that there is a blunted response to loading from increasing BMI or adiposity in the older cohort (i.e. females aged between 50-80 years old). The implication of this finding suggests that the slope of the regression in the Lafortuna study (2013) maybe artificially lower, providing erroneous conclusions.

A limitation of the current thesis chapter regarding investigations into the effect of body composition on both muscle volume and PCSA is through not controlling for intra muscular adipose tissue (IMAT), which is evident to varying degrees in both obese (Hilton et al. 2008) and young/ old populations (Kent-Braun et al. 2000). Kent-Braun et al. (2000) demonstrated significant differences between the non-contractile component of the tibialis anterior between young and old females. If utilising data from this study as a standard for assessing the non-contractile component of the GM, the muscle tissue content values may have been over estimated by 6% in young females and 13.9% in older females (Kent-Braun et al. 2000). However, if values were used as a standard from the Kent-Braun et al. (2000) study, this still may underestimate the true value of IMAT due to obese individuals possessing greater levels of IMAT than normal weight controls (Hilton et al. 2008). In regards to the potential effect this may have on the regression

analyses reported in chapter 4, from the inferred age related muscle content loss in the obese, would therefore be underestimated. This would be due to an increase in both non-contractile tissue associated in ageing (Kent-Braun et al. 2000) and through increasing BMI (Hilton et al. 2008) of which would create an increase in the steepness of the slope of the linear regression. These differences would also have potential implications for both the magnitude of intrinsic strength (nMVC/GM muscle volume) and GM specific force, based on the overestimation of GM muscle volume and PCSA. In relevant terms this would mean that both the obese young and old participants having potentially greater skeletal muscle intrinsic capacity than the values reported in both chapter 4 and 5. This effect could potentially remove any significant differences in lower intrinsic muscle strength reported in the young female participants in chapter 4 after controlling for IMAT. Therefore, implications are for further research to systematically quantify IMAT in the calculation of muscle volume, due to the potential cascade effect this has upon underestimating intrinsic and specific force.

Chapter 4 investigated the effect of body composition on force at both a gross (nMVC/GM muscle volume i.e. intrinsic strength) and fascicle (i.e. specific force) level. The study revealed between group differences, when force was quantified at a gross level, between the obese females, whether obesity was classified by BMI (-26%; p=0.007) or adiposity (-11%; p=0.025) against their normal weight counterparts. These findings differ from previous research that reported no between group differences when force was normalised to fat free mass (Maffiuletti et al. 2007) or anatomical cross sectional area (ACSA) (Blimkie et al. 1990). The aim of this thesis, as stated previously, was to control for physiological and biomechanical determinants of muscle strength, thus improve the accuracy of measures when quantifying parameters such as muscle size through measuring muscle volume in comparison to measures such as ACSA (Akagi et al. 2009). Interestingly at a fascicular level, between group differences were removed irrespective of whether obesity was classified by BMI or adiposity in both a young and old cohort. This is the first study to classify the effect of BMI or adiposity on specific force, so there was no data to compare against in this population. However, the mean values reported in all BMI classifications were slightly higher to that reported by Morse et al. (2008) in the gastrocnemius lateralis of adolescent boys and adult males, with a wide range of BMI.

Within this chapter, the impact of BMI and/or adiposity on the magnitude of ageing-related sarcopenia and asthenia was quantified in variables of nMVC, GM

muscle volume, intrinsic strength and GM specific force. The greatest deleterious changes were observed in females categorised obese by either adiposity or BMI in the accelerated loss of nMVC and GM muscle volume with age. A suggested mechanism that can potentially underpin the faster loss of muscle mass may be the cumulative effect of higher inflammation observed in both obese and elderly individuals (Schrager et al. 2007; Park et al. 2005; Degens 2010; Cesari et al. 2004), coupled with a lower anabolic profile both in old age (Bucci et al. 2013) and obesity (Galli et al. 2012; Frost et al. 2003). These findings enhance the susceptibility to sarcopenia in the obese, whilst emphasising the deleterious impact of this condition. These findings were mirrored in a male population as documented in chapter 5 and in chapter 6 focusing on gender-related variations in the adaptation response from obesity. However, the effect of ageing on nMVC loss in both obese male and obese female cohorts has specific importance in old individuals irrespective of gender. As further exacerbation of an existing relatively low 'strength to body mass' ratio in obese individuals (documented in chapter 2) may render daily functional capacity even more compromised, with the ability to carry out tasks such as rising from a chair or squatting deep to reach items on the floor for instance, quicker to lose.

Arguably, a more detailed description of decade-by-decade changes on sensitivity to adiposity would be warranted in future studies, using a longitudinal study design, as would the additional consideration of ethnicity, two topics on which we expand below. The rationale behind a more detailed description into decade-by-decade changes is due to the reported loss of physical functioning at the onset of the menopause in females (Sowers et al. 2007). Future research should therefore examine if obesity exacerbates the decrement in physical functioning at key age groups in female cohorts. Expansion of such a study should also lead to examining if there is an existence of a functional threshold of strength in the obese. Previous research (Ploutz-Snyder et al. 2002) has reported a threshold of <3.0 Nm/kg in the quadriceps of elderly individuals for an increased risk of having functional limitations such as rising from a chair, gait speed and ascending and descending stairs. However after analysis of the previous literature, this research has not been applied to an elderly obese population specifically in the plantar flexors, which has functional relevance due to the major role this muscle group plays in postural balance (Onambele et al. 2006a). Therefore, the identification of both a functional threshold that is gender specific would allow for targeted and individualised programs to be applied in later life ensuring that quality

of life is not impacted upon, through minimising the risk of developing the majority of functional impairments. Future work may also consider controlling for ethnicity, due to the greater susceptibility of developing diabetes among Asians, Hispanics, and blacks when compared against white individuals, even after taking into account differences in BMI (Shai et al. 2006). The increased susceptibility of developing diabetes in these ethnic cohorts could potentially exacerbate the effects of obesity through the potential development of peripheral neuropathy and loss of muscle strength through motor weakness (Andersen et al. 1996) (implications discussed previously).

Finally examination of chapter 5 and 6 revealed the impact of the continuum of adiposity (and/or BMI) to be similar between a male and female population, except at the fascicular level. The slopes of the regressions indirectly indicated that the young male cohort increases specific force with either increasing BMI or total body adiposity in comparison to the slope seen in the female cohort. These differences at the fascicular level were continued when examining gender variations on the impact of BMI and/or adiposity on the magnitude of ageing-related sarcopenia and asthenia. The slopes of the regressions indirectly implied the obese male individuals to lose GM specific force at a steeper rate than their female obese counterparts. However, due to the smaller sample size in the male cohort (n=34) vs. the female cohort (n=94), future research should aim to increase the sample size of the male cohort to confirm or reject the conclusions derived from this thesis.

## Implications of findings and future research

Whilst overall body mass (chapter 3) has been shown to be the strongest predictor associated with increased skeletal muscle strength and size in an untrained population, adiposity has been revealed as the factor that negatively impacts on the neuromuscular determinants of maximum strength (chapter 2) and exacerbates the age-related loss of muscle content observed in ageing (chapters 4 & 5). The implications of these findings form the basis for the rationale to why obesity is associated with functional impairments such as mobility limitations, rising from a chair and difficulty climbing up and going down stairs (Rolland et al. 2009). The accelerated loss of muscle content with age in the obese is highly relevant to the exacerbation in muscle torque loss observed in this cohort, due to direct association between muscle size and strength (Close 1972). Therefore exacerbation of muscle weakness already observed in old age would increase the

associated risk of falls (Campbell et al. 1989), mobility limitations (Visser et al. 2005) and bone fractures (Aniansson et al. 1984), leading to a condition coined 'osteosarcopenic obesity' (Ormsbee et al. 2014; Ilich et al. 2014). Lower maximal strength has been previously explained through lower physical activity status observed in the elderly, through (Myint and Welch 2012) the reduction of agonist muscle activation, lower muscle mass and FPA (Morse et al. 2004). This study controlled for physical activity status through using a 'health and activity' questionnaire (see appendix for questionnaire). The questionnaire quantified 3 different areas of physical activity, including work, sport and leisure. Physical activity was not found to be a confounding factor from the scores reported in the activity questionnaire (see appendix pages 212-214 for scores). Interestingly, current recommendations for healthier ageing are to increase the amount of physical activity an individual undertakes (Myint and Welch 2012). However, a confounding factor not suggested by Myint and Welch (2012) is through decreasing the amount of time an individual spends being sedentary. Sedentary behaviour is classified through both the amount of time an individual spends in a sitting/reclined position and has an energy expenditure level below 1.5 METs (Sedentary Behaviour Research 2012) and in addition low levels of sendentarism has been independently associated with healthy ageing (Dogra and Stathokostas 2012). The questionnaires utilised in this thesis factored in the amount of time spent sitting during work and the amount of time spent watching television, that have been noted as sedentary behaviours, yet we found no group differences either by age or body composition grouping. Our observations are therefore that we had a particularly 'active' study sample. In a different sample to ours where the level of sedentary behaviour may be significantly higher in the obese (as one would tend to expect (Chastin et al. 2012)), this may have negative implications not only on skeletal muscle, but additionally on bone mineral density (Chastin et al. 2014).

Therefore, even though the self-reporting physical activity questionnaire utilised in this study has been validated against physical activity status (Baecke et al. 1982), a more stringent method of quantifying the daily metabolic demands of obese young and old individuals would have merit when investigating differences observed in skeletal muscle characteristics between BMI/adiposity classifications due to the poor association between self reported physical activity and activity recording devices (Prince et al. 2008).

Methodologies that may be utilised are Global Positioning System (GPS) devices detailing distances covered, speeds undertaken and accompanying heart rate data already observed in quantifying the metabolic demands of elite sport (Cunniffe et al. 2009) and previously utilised devices in quantifying sedentarism such as 3D-accelerometry (Chastin et al. 2014). These devices may also be utilised to examine the effects of loaded exercise upon an obese individual, prior to and completing an exercise intervention. This maybe be of interest in the same way that weighted exercise using loaded vests (e.g. 15% body weight) has shown positive effects on bone turnover and isokinetic strength, for instance in post-menopausal women (Klentrou et al. 2007).

Accompanying any potential differences in physical activity status and levels of sedentary behaviour between obese and normal weight young and old individuals is the suggestion of being continuously in a higher state of background endocrine inflammation, which has been previously demonstrated in an obese ageing population (Schrager et al. 2007; Visser et al. 2002), and forms part of further investigations currently being undertaken in our research group. Thus, our endocrine data mining will aim to elucidate the role that inflammation has in the obese young and old, with regard to negatively effecting muscle strength and size (Barbieri et al. 2003). Pro-inflammatory cytokines that would be of interest are interlukin-6 and tumour necrosis factor α. However, also examining the roles of insulin like growth factor-1, transforming growth factor β, testosterone and oestrogen in potentially explaining the limited increases shown in muscle volume in the obese elderly would be of interest.

Finally yet importantly, knowledge of the time spent by the individual in being obese (i.e. the duration of their obese status at the point of testing) may also present a different facet to the adiposity-sarcopenia-asthenia triad. However, this data would be difficult to ascertain due to the study being cross-sectional in design and not longitudinal based, whilst in addition any information would be self reported by the individual and therefore not necessarily reliable.

A limitation of this thesis as mentioned previously in both chapter 5 and the general discussion is the lower sample number of male participants. Therefore, the conclusions derived from both the younger and older male sample need to be confirmed through future work with a greater male sample that is equal to their female cohorts utilised within this thesis, since the power of the female studies was adequate at $\beta \geq 0.8$."

## Conclusion

The main findings of this thesis demonstrate that obesity/high adiposity acts as a loading stimulus to a differing extent in the antigravity muscles of the lower limb in young versus old populations. However within chapter 2, obesity defined by adiposity (Baumgartner et al. 1998), appeared to significantly lower one of the neuromuscular determinants of strength, namely agonist muscle activation. This finding has only previously reported in an adolescent population (Blimkie et al. 1990) and had not been investigated until now in either a young adult or an older adult population. The reality of compromised muscle activation in the obese is in terms of the exacerbation of an already poor 'strength to body mass' ratio, making habitual functional tasks difficult, especially in older obese individuals. In addition to the negative aspect of impaired muscle activation, old obese females do not exhibit an increased muscle volume (relative to their other BMI-classified counterparts), despite the loading stimulus of adiposity, thus decreasing their net maximum force capability. This is further demonstrated through the increased loss of muscle content with ageing in the obese male and female populations.

Therefore, separate conclusions can be drawn on the impact of obesity on muscle structure and function between young and old individuals. Obesity in the young is detrimental with regards to limiting maximal force generation and having lower maximum strength relative to body mass, but skeletal muscle is shown to adapt to carrying the extra inert mass of adiposity through an increase in both FPA and PCSA. This conclusion does not apply within an older age classification, as supplemented with lower muscle activation; the muscle fails to increase its volume, therefore not allowing an individual greater maximum torque capability. We would hypothesise that an environment of inflammation evident in obesity and ageing (Schrager et al. 2007; Park et al. 2005; Degens 2010; Cesari et al. 2004) may underlay the summation of detrimental effects. Implications of this research to a wider audience, lead towards decreasing adiposity in advancing age as a priority in order to decrease the risk of functional limitations associated with obesity in old age (Rolland et al. 2009). Further research should focus on the effect of sedentary behaviour has in accompaniment with increased adiposity evident in the obese and any link to successful ageing.

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# Appendices

## Publications

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# Combined effects of body composition and ageing on joint torque, muscle activation and co-contraction in sedentary women

**D. J. Tomlinson · R. M. Erskine · C. I. Morse · K. Winwood · G. L. Onambélé-Pearson**

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**Abstract** This study aimed to establish the interplay between body mass, adiposity, ageing and determinants of skeletal muscle strength. One hundred and two untrained healthy women categorised by age into young (Y) (mean ± SD, 26.7 ± 9.4 years) vs. old (O) (65.1 ± 7.2 years) were assessed for body fat, lean mass, plantar flexion and dorsiflexion maximum voluntary isometric contraction (MVC) torque, muscle activation capacity and antagonist muscle co-contraction. MVC torque normalised to body mass in the obese group was 35 and 29 % lower ($p<0.05$) in Y and 34 and 31 % lower ($p<0.05$) in O, compared with underweight and normal weight individuals, respectively. Y with ≥40 % body fat had significantly lower activation than Y with <40 % body fat (88.3 vs. 94.4 %, $p<0.05$), but O did not exhibit this effect. Co-contraction was affected by ageing (16.1 % in O vs. 13.8 % in Y, $p<0.05$) but not body composition. There were significant associations between markers of body composition, age, strength and activation capacity, with the strongest correlation between muscle strength and total body mass ($r^2=0.508$ in Y, $p<0.001$, vs. $r^2=0.204$ in O, $p<0.01$). Furthermore, the age-related loss in plantar flexion (PF) MVC torque was exacerbated in obese compared to underweight, normal weight and overweight individuals (−0.96 vs. −0.54, −0.57 and −0.57 % per year, $p<0.05$). The negative impact of adiposity on muscle performance is associated with not only muscular but also neural factors. Overall, the effects of ageing and obesity on this system are somewhat cumulative.

**Keywords** Activation · Adiposity · Ageing · Lean mass · Muscle strength · Obesity

D. J. Tomlinson · R. M. Erskine · C. I. Morse · K. Winwood · G. L. Onambélé-Pearson (✉)
Institute for Performance Research, Department of Exercise and Sport Science, Manchester Metropolitan University, Crewe Green Road, Crewe CW1 5DU, UK
e-mail: g.pearson@mmu.ac.uk

*Present Address:*
R. M. Erskine
Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UK

## Introduction

Obesity is associated with high body fat and several co-morbidities including lowered functional mobility, particularly in the elderly (Zoico et al. 2004). The latter effect is likely linked to decreased strength to body mass ratio in obese compared with normal weight individuals in both young (Maffiuletti et al. 2007) and aged populations (Rolland et al. 2004). Contributors to strength, over and above muscle tissue content (Erskine et al. 2010a), are agonist muscle activation (Morse et al. 2004), antagonist co-contraction (Klein et al. 2001) and tendon moment arm (Erskine et al. 2010a). Yet, the link between obesity and the principal factors contributing to decreased strength to body mass ratio in both young and ageing populations has yet to be explored. The combined impact of ageing and obesity is of particular interest, as strength is known to decline with

ageing (Morse et al. 2005), yet it remains to be seen what effect, if any, increased adiposity has on the ageing-related sarcopenia and in particular on the neuromuscular components of muscle strength.

It could be hypothesised that obesity in both young and old persons would load the antigravity muscles causing hypertrophy, in a manner similar to being placed in a hypergravity environment (Maffiuletti et al. 2007, 2013; Blimkie et al. 1990). Indeed, 3 weeks of simulated hypergravity in young athletes wearing a weighted vest ranging from 7 to 13 % of body weight from morning until night increased participants' muscle power (Bosco et al. 1984, 1986; Bosco 1985). Interestingly, similar increases in muscle power were demonstrated in postmenopausal women who utilised weighted vests (Kemmler et al. 2007). When relating this to an obese individual who has carried excess weight continuously for years, not weeks, strength gains may follow those seen with resistance training, thus having both neural and muscular underpinnings (Erskine et al. 2010b). Although the loading intensity of increased adiposity is not as high in the conventional resistance training regimes, the volume of loading is likely to be higher with lower loads being lifted during 'repetitions' of daily tasks and over longer periods of time. In support of this hypothesis, low-load, high-volume resistance exercise has been shown to stimulate muscle protein synthesis more than traditional high-load, low-volume exercise (Burd et al. 2010).

In terms of the muscular factors of decreased strength, muscle strength is 24.2 and 22.2 % higher (absolute torque or torque normalised to thigh muscle mass, respectively) in obese compared to non-obese adolescent boys (Abdelmoula et al. 2012). In contrast, lower limb maximum voluntary contraction (MVC) torque and power (both absolute and normalised to muscle volume) is lower in obese compared to non-obese persons (Hilton et al. 2008). In terms of the neural factors of decreased strength, one limiting factor could be the central drive. However, studies report that increased adiposity is in fact associated with increased neural sympathetic drive (Alvarez et al. 2002). This would therefore suggest that the deleterious effects of weakness associated with high fat mass are muscular but not neural in origin. However, a study reported a decrease in the muscle activation capacity (85.1 vs. 95.3 %) in obese compared to non-obese adolescent males (Blimkie et al. 1990). Unfortunately, exercise training status was not monitored in this study, and this could account for differences in muscle activation (Hakkinen et al. 1998). In another study, it was found that obese individuals had greater fat-free mass yet similar strength values compared to their normal weight counterparts (Rolland et al. 2004), hence further supporting the suggestion of obesity-related weakness having a neural basis (e.g. decreased agonist activation capacity). Moreover, whilst the expectation is that with increased age, there will be a decrease in agonist activation capacity even in the absence of obesity (Morse et al. 2004), it is unclear whether the above-described decreased activation capacity seen in the adolescents would be mirrored in adults and/or exacerbated in the elderly.

Indeed, skeletal muscle ageing has been well documented and is characterised by lower muscle strength (Morse et al. 2005; Onambele et al. 2006a, b), decreased muscle volume (Thom et al. 2005), decreased agonist activation (Morse et al. 2004) and increased antagonist co-contraction (Klein et al. 2001). The consensus is that many of these ageing effects are caused through decreased habitual physical activity levels and sarcopenia (Rosenberg 1997). Hence, since there is a recognised age-related prevalence in increased body fat percentage termed sarcopenic obesity (Zamboni et al. 2008), characterised by a combination of reduced skeletal muscle mass and increased intramuscular fat (Hilton et al. 2008), obesity would further compound the ageing effects. In support of this hypothesis, sarcopenic obesity would be expected to aggravate the impact of ageing on physical functions including stair climbing, rising from a chair and lifting objects (Rolland et al. 2009). However, Rolland and colleagues did not quantify either agonist muscle activation capacity or antagonist co-contraction (Rolland et al. 2004), thus potentially masking the true impact of sarcopenic obesity.

The present study therefore aimed to contrast how different levels of BMI vs. lean muscle mass vs. adiposity impact on both muscular (absolute and normalised MVC ankle joint torque) and neural factors (agonist muscle activation, antagonist co-contraction) underlying skeletal muscle function. The study also aimed to determine whether the effects of ageing and adiposity were additive. It was hypothesised that: (1) absolute torque in both young and old obese individuals would be higher, but torque relative to body mass lower, compared to underweight, normal weight and overweight individuals; (2) muscle activation would be significantly lower in obese young and old individuals; and (3) the

slope of the relationship between adiposity and joint torque, or activation capacity, would be steeper in the older individuals relative to their younger counterparts.

## Method

### Participants

Untrained females (*n*=102) categorised by age into young (Y): 18–49 years old or old (O): 50–80 years old volunteered to take part in this study. Participants were sub-categorised into four body mass index classifications (BMI—body mass (kg)/stature$^2$ (m)) into *underweight* (BMI <20), *normal* (BMI 20–24.9), *overweight* (BMI 25–29.9) and *obese* (BMI >30). Group information on age, stature and body mass is presented in Table 1. Participants were excluded if there was any issue with lower limb muscles/joints affecting their mobility or ability to exert maximum force. Fasted blood glucose levels were used as an indication of undisclosed peripheral neuropathy, a condition which has a detrimental effect on force production (Hilton et al. 2009).

Ethical approval was obtained from the local ethics committee, and all participants gave their written informed consent prior to undertaking any assessment.

### Body composition measure

Body composition analysis (body content of fat, lean muscle and bone) was performed using a dual-energy X-ray absorptiometry (DEXA) scanner (Hologic Discovery: Vertec Scientific Ltd, UK) with participants fasted for 12 h prior to scanning. Participants were laid in a supine position throughout the 7-min scanning procedure. Segmental analysis of the whole body scan provided quantification for lean leg mass, later used in the normalisation of the joint torque data. The android to gynoid ratio (A/G) of each participant was calculated using the Hologic APEX software (version 3.3). The android region was classified as the area between the mid-point of the lumbar spine to the top of the pelvis, whilst the gynoid region was classified as the area between the head of the femur and mid-thigh.

**Table 1** Descriptive variables for BMI classifications in both young and old age classifications. The A/G ratio (i.e. android/gynoid dimensional comparison or waist-hip ratio), where it is >1.00, is utilised as an indicator of increased risk of cardiovascular disease (Folsom et al. 2000)

| Young (18–49) | Underweight (*n*=13) | Normal (*n*=13) | Overweight (*n*=10) | Obese (*n*=18) | BMI effect | Ageing effect |
|---|---|---|---|---|---|---|
| Age (years) | 23.0 (6.7) | 23.2 (7.9) | 23.6 (8.0) | 36.9 (10.7) | $p$=0.002 | $p$=0.001 |
| Height (cm) | 167.3 (4.7) | 164.1 (8.6) | 162.8 (7.4) | 166.3 (7.6) | $p$=0.422 | $p$=0.002 |
| Body mass (kg) | 52.7 (3.9) | 58.3 (6.3) | 74.6 (8.3) | 97.3 (13.1) | $p$<0.001 | $p$=0.683 |
| BMI (kg/m$^2$) | 18.8 (0.9) | 21.6 (1.1) | 28.1 (2.4) | 35.2 (4.4) | $p$<0.001 | $p$=0.625 |
| Body fat (%) | 26.3 (3.9) | 36.4 (5.1) | 38.7 (3.9) | 45.3 (3.9) | $p$<0.001 | $p$=0.001 |
| Total body fat (kg) | 13.7 (2.2) | 17.2 (2.7) | 28.5 (6.8) | 45.2 (7.3) | $p$<0.001 | $p$=0.376 |
| Total lean mass (kg) | 35.7 (3.4) | 37.2 (4.7) | 42.6 (4.3) | 49.4 (7.0) | $p$<0.001 | $p$=0.002 |
| Fat mass leg (kg) | 3.2 (0.5) | 3.9 (0.6) | 5.8 (1.8) | 7.7 (1.3) | $p$<0.001 | $p$=0.859 |
| A/G ratio | 0.70 (0.09) | 0.77 (0.12) | 0.95 (0.14) | 1.06 (0.08) | $p$<0.001 | $p$=0.062 |
| **Old (50–78)** | **Underweight (*n*=4)** | **Normal (*n*=13)** | **Overweight (*n*=18)** | **Obese (*n*=11)** | **BMI effect** | **Ageing effect** |
| Age (years) | 63.8 (5.7) | 63.5 (7.7) | 68.2 (4.8) | 62.5 (9.0) | $p$=0.143 | $p$=0.001 |
| Height (cm) | 159.1 (5.3) | 159.4 (5.2) | 162.1 (5.8) | 162.5 (5.7) | $p$=0.268 | $p$=0.002 |
| Body mass (kg) | 48.4 (4.2) | 56.6 (8.5) | 71.6 (8.5) | 90.1 (16.4) | $p$<0.001 | $p$=0.683 |
| BMI (kg/m$^2$) | 19.1 (0.8) | 22.2 (1.0) | 27.3 (1.2) | 34.1 (5.7) | $p$<0.001 | $p$=0.625 |
| Body fat (%) | 26.5 (2.1) | 36.0 (3.6) | 42.9 (3.3) | 46.1 (3.0) | $p$<0.001 | $p$=0.001 |
| Total body fat (kg) | 12.5 (2.8) | 19.9 (2.9) | 29.8 (3.4) | 40.9 (11.3) | $p$<0.001 | $p$=0.376 |
| Total lean mass (kg) | 32.8 (2.4) | 33.3 (2.8) | 37.4 (2.4) | 44.7 (6.7) | $p$<0.001 | $p$=0.002 |
| Fat mass leg (kg) | 2.8 (0.3) | 3.8 (0.7) | 5.6 (0.1) | 6.5 (2.0) | $p$<0.001 | $p$=0.859 |
| A/G ratio | 0.66 (0.09) | 0.89 (0.16) | 0.97 (0.11) | 1.10 (0.08) | $p$<0.001 | $p$=0.062 |

Data are presented as mean ± SD

### Muscle strength

Plantar flexion (PF) and dorsiflexion (DF) MVC torque was assessed in the dominant limb using an isokinetic dynamometer (Cybex Norm, Cybex International, New York, NY, USA). Participants were seated with a hip angle of 85° and their dominant leg fully extended. The dominant foot was secured to the footplate of the dynamometer using inextensible straps, ensuring the lateral malleolus was aligned with the centre of rotation. Participants were firmly strapped at the hip, distal thigh and chest with inextensible straps to minimise movement. Prior to undertaking any MVCs, the participants completed a series of warm-up PF and DF contractions.

Participants subsequently performed four isometric PF (×2) and DF (×2) MVCs with the ankle positioned at 0° (anatomically neutral), with 1–2 min of rest between efforts. MVCs were repeated if there was >10 % difference between MVCs to ensure true MVC was obtained. The highest recorded PF and DF MVCs were used for subsequent analysis. Surface electromyography (EMG) of the tibialis anterior (TA) was recorded during all MVCs to calculate antagonist muscle co-contraction during PF MVC (see below for details). Verbal encouragement and biofeedback were provided during each effort.

### Antagonist muscle co-contraction

EMG (using pre-gelled unipolar Ag:AgCl electrodes (Medicost, Denmark)) was used to assess muscle co-contraction of the tibialis anterior during PF MVC. Two electrodes (skin contact size: 30 mm × 22 mm) were placed proximally at one third of the tibialis anterior muscle length, mid muscle belly, with a 1–2 mm gap separating each electrode. A reference electrode (Medicost, Denmark) was placed on the head of the fibula. Raw EMG was then recorded at 2,000 Hz, with the band pass filter set at 10–500 Hz and notch at 50 Hz.

Muscle co-contraction (%) was calculated at 0° ankle angle utilising the raw EMG signal (computed as root mean square (RMS) 500 ms either side of the instantaneous peak torque) of the tibialis anterior during PF MVC divided by EMG during DF MVC. Co-contraction torque (Nm) was the product of percent co-contraction and maximal DF torque.

Hence, net PF MVC torque was calculated as the sum of observed maximal PF torque and co-contraction torque. This method assumes that the DF EMG/torque relationship is linear (Maganaris et al. 1998).

### Muscle activation

PF agonist muscle activation was estimated using the interpolated twitch technique (Morse et al. 2004; Pearson and Onambele 2006). Briefly, percutaneous stimuli (DSV Digitimer Stimulator, Digitimer, Herts., UK) were applied to the gastrocnemius using rubber stimulation pads (50 mm × 100 mm; American Imex, Irvine, CA, USA). The two stimulation pads were placed transversely distal to the popliteal crease and myotendinous junction of the soleus. The amplitude of the stimuli was determined prior to interpolation whilst the participant was in a relaxed state, administering twitches starting from 50 mA, with subsequent increments of 50–100 mA, until no further increase in twitch torque was elicited. The assessed supramaximal doublets (i.e. the stimulus intensity above which no further increase in doublet torque was observed with increased stimulus intensity) were superimposed during a maximal PF MVC. The calculation used to establish muscle activation (%) is shown below:

$$(\text{Superimposed doublet torque}/\text{resting doublet torque}) \times 100 = \text{muscle activation}(\%)$$

### Statistical analyses

Statistical analyses were carried out using SPSS (version 19, SPSS Inc., Chicago, IL, USA). To determine parametricity, Kolmogorov-Smirnov or Shapiro-Wilk (normal distribution) and Levene's tests (homogeneity of variance) were utilised. If parametric assumptions were met, a factorial 2 × 4 ANOVA (age × BMI) was utilised with post hoc Bonferroni correction for pairwise comparisons. Where parametric assumptions were breached, Mann-Whitney *U* or Kruskal-Wallis *H* was utilised. Pearson correlations described the relationships between PF MVC and leg lean mass, muscle activation, body mass, fat mass, total lean mass, body fat%, BMI

and A/G ratio. Additionally, linear and multiple regressions were used to determine the best predictors of PF MVC. A comparison of the regression coefficients and slopes was conducted using *z* transformations and Student's *t* statistic. Data are reported as mean±SD, and statistical significance was accepted when $p \leq 0.05$. Study power ($\beta$) and effect size ($pc^2$) are also reported.

## Results

### Body composition

Table 1 displays the descriptive values for age, height, body mass, BMI, body fat%, total body fat, total lean mass, leg fat mass and A/G ratio for Y and O females categorised by BMI. A 2×4 factorial ANOVA of body fat% revealed a main effect for age ($p<0.001$; $pc^2=0.164$; $\beta=0.904$) and for BMI ($p<0.001$; $pc^2=0.736$; $\beta=1.000$) and an age×BMI interaction ($p=0.029$; $pc^2=0.091$; $\beta=0.713$).

A Mann-Whitney test on leg lean mass revealed a main effect of age ($p<0.001$), whilst a Kruskal-Wallis test revealed between-group differences for leg lean mass between classifications in Y ($p<0.001$) and O ($p<0.001$). However, Y obese had 28 and 27 % more leg lean mass than Y underweight ($p<0.001$) and Y normal weight ($p<0.001$) individuals, respectively, whilst obese O had 26, 27 and 20 % more leg lean mass than O underweight ($p=0.018$), O normal weight ($p<0.001$) and O overweight ($p=0.04$), respectively (Table 4).

There were strong positive correlations ($p<0.001$) between leg lean mass and body mass, and lean mass and body fat, in both Y and O groups (Tables 2 and 3). Ageing affected neither the degree of association in these correlations ($p>0.05$) nor the slope of the regressions ($p<0.05$, Table 3). In predicting leg lean mass, a stepwise multiple linear regression was conducted with variables body fat%, body mass, lean mass, fat mass and android to gynoid ratio in all individuals. Total lean mass and body fat% were the only predictors in a stable model, which, in combination, explained 93 % of the leg lean mass in both Y and O individuals ($p<0.001$; $r=0.966$).

### Muscle strength

A 2×4 factorial ANOVA on PF MVC torque revealed a main effect for age ($p<0.001$; $pc^2=0.327$; $\beta=1.000$) and a main effect for BMI ($p=0.001$; $pc^2=0.252$; $\beta=0.937$), yet there was no significant age×BMI interaction ($p=0.676$; $pc^2=0.016$; $\beta=0.151$). However, Y obese had 23 and 20 % higher uncorrected PF MVC torque than underweight ($p<0.008$) and normal weight ($p=0.039$) individuals, whilst O individuals revealed no significant between-group differences ($p>0.05$) (Table 4).

PF MVC torque relative to body mass was higher in Y vs. O ($p<0.001$; $pc^2=0.291$; $\beta=1.000$) and differed according to BMI ($p<0.001$; $pc^2=0.285$; $\beta=1.000$), but there was no significant age×BMI interaction ($p=0.410$; $pc^2=0.030$; $\beta=0.258$). However, Y obese had 43, 35 and 25 % lower PF MVC torque relative to body mass than Y underweight ($p<0.001$), Y normal weight ($p<0.001$) and Y overweight ($p=0.031$), respectively, whilst obese O exhibited 43 % lower uncorrected PF MVC torque relative to body mass than O underweight ($p=0.032$) (Table 4).

The net PF MVC torque revealed a main effect of age ($p<0.001$; $pc^2=0.293$; $\beta=1.000$) and BMI ($p<0.001$; $pc^2=0.224$; $\beta=0.993$), but no significant age×BMI interaction ($p=0.581$; $pc^2=0.021$; $\beta=0.184$). However, Y

**Table 2** Linear regressions ($r^2$) between net PF torque, leg lean mass and agonist muscle activation against a series of descriptive variables in young and old untrained females

| | Young ($n=34$) | | | Old ($n=40$) | | |
|---|---|---|---|---|---|---|
| | PF MVC | Leg lean mass | Activation | PF MVC | Leg lean mass | Activation |
| Leg lean mass | 0.621*** | – | 0.176** | 0.36*** | – | NS |
| Body mass | 0.508*** | 0.749*** | 0.164** | 0.266** | 0.677*** | NS |
| Fat mass | 0.383*** | 0.536*** | 0.157** | 0.155* | 0.472*** | NS |
| Lean mass | 0.600*** | 0.936*** | 0.125** | 0.242*** | 0.984*** | NS |
| Body fat% | 0.261** | 0.240*** | 0.103* | NS | 0.135* | NS |
| BMI | 0.411*** | 0.548*** | 0.176** | 0.157** | 0.559*** | NS |

*$p<0.05$; **$p<0.01$; ***$p<0.001$

**Table 3** Pearson correlations, *z* transformation of *r* and Student's *t* statistic between net PF MVC and leg lean mass against a series of descriptive variables in young and old untrained females

| | Young | | | Old | | | Correlation coefficient | Ageing effect |
|---|---|---|---|---|---|---|---|---|
| | *n* | *r* value | slope | *n* | *r* value | slope | *z* transformation of *r* | Student's *t* statistic |
| PF MVC vs. leg lean mass | 54 | 0.79*** | 19.63 | 48 | 0.51*** | 12.48 | 1.93 | 1.90 |
| PF MVC vs. body mass | 54 | 0.71*** | 1.26 | 48 | 0.45** | 0.75 | 1.70 | 1.85 |
| PF MVC vs. fat mass | 54 | 0.62*** | 1.70 | 48 | 0.37* | 0.92 | 1.57 | 1.70 |
| PF MVC vs. lean mass | 54 | 0.78*** | 3.70 | 48 | 0.49*** | 2.28 | 1.93 | 1.94 |
| Log HNet vs. BM | 54 | 0.87*** | 0.06 | 48 | 0.83*** | 0.06 | 0.45 | 0.77 |
| Log HNet vs. FM | 54 | 0.75*** | 0.08 | 48 | 0.69*** | 0.07 | 0.33 | 0.74 |
| Log HNet vs. lean mass | 54 | 0.97*** | 0.19 | 48 | 0.93*** | 0.18 | 0.50 | 0.44 |

*p<0.05; **p<0.01; ***p<0.001 (If $|z| > 1.96$, $p<0.05$; $|z| > 2.58$, $p<0.01$) (Student's *t* statistic significance if *t* falls outside ±1.96, $p<0.05$)

obese had 27 and 23 % higher corrected PF MVC torque than Y underweight ($p<0.001$) and Y normal weight ($p=0.002$) individuals, whilst O revealed no significant between-group differences ($p>0.05$) (Table 4).

Net PF MVC torque/body mass revealed a main effect of age ($p<0.001$; $pc^2=0.281$; $\beta=1.000$) and BMI ($p<0.001$; $pc^2=0.280$; $\beta=1.000$), but no significant age × BMI interaction ($p=0.821$; $pc^2=0.010$; $\beta=0.107$). However, Y obese had 35 and 29 % lower net PF MVC torque/body mass than Y underweight ($p<0.001$) and Y normal weight ($p<0.001$) individuals, whilst obese O had 31 % lower net PF MVC torque/body mass than O normal weight ($p=0.016$) (Table 4).

There were strong positive correlations ($p<0.001$) between net PF MVC torque and body mass, lean mass and body fat in both Y and O groups (as seen in Table 2). Ageing affected neither the degree of association in these correlations ($p>0.05$) nor the slope of the regressions ($p>0.05$, Table 3). In predicting net PF MVC, a stepwise multiple linear regression was conducted with independent variables being body fat%, body mass, lean mass, fat mass and android to gynoid ratio, for the pooled study population (i.e. all Y and O data). Total lean mass was the only predictor in the stable model and explained 51 % of net PF MVC, and body mass normalised torque regardless of age ($p<0.001$; $r=0.710$).

Net PF MVC and leg lean mass were correlated in both the Y ($p<0.001$; $r^2=0.625$) and O ($p<0.001$; $r^2=0.260$) age groups, with similar slopes in the two age groups (Table 3). Figure 1 displays the percent change per 10 years (assuming % change is linear) in muscle loss, PF torque, net PF torque and net PF torque normalised for BM categorised by BMI.

### Muscle co-contraction

There was no significant difference in antagonist co-contraction between BMI groups ($p=0.730$) (Table 4). Yet, there was an effect of age, with O exhibiting lower co-activation than Y (16.1 vs. 13.8 %; $p=0.042$).

### Voluntary muscle activation

Muscle activation did not differ between BMI groups in either Y ($p=0.138$) or O ($p=0.701$) (Table 4). When Y and O were categorised by body fat% (Fig. 2), muscle activation was higher in Y low body fat% (BF%) than Y high BF% ($p=0.019$) but there was no difference in O ($p=0.458$) (Fig. 1). Y also demonstrated higher muscle activation capacity than O (92 vs. 84.5 %; $p=0.001$).

## Discussion

Our data have provided supportive evidence for our hypothesis that absolute torque in young obese individuals would be higher compared to that of underweight and normal weight individuals. However, the old obese females did not exhibit significantly higher absolute torque compared to the other three BMI classifications. Unexpectedly, when torque was normalised to body mass, the young obese were significantly weaker than the other three BMI classifications, whereas the old obese were only weaker than their old underweight counterparts, thereby suggesting a somewhat protective impact of obesity for the old, or at least, this support that

**Table 4** Displays strength data (FF torque-test PF torque-control) of eight muscle activations and antagonist co-contractions (agonist activation + control) divided by knee mean as body-young and -old BMI classifications

| | Young | | | Old | | | | Young BMI effect | Old BMI effect | Ageing effect |
|---|---|---|---|---|---|---|---|---|---|---|
| | Underweight (*n*=11) | Normal (*n*=15) | Overweight (*n*=16) | Obese (*n*=10) | Underweight (*n*=4) | Normal (*n*=5) | Overweight (*n*=16) | Obese (*n*=11) | | | |
| PF torque (Nm) | | | | | | | | | | | |
| FF MVC (F | 125.7 (22.1) | 131.6 (29.1) | 150.6 (34.8) | 161.7 (37.7) | 94.8 (32.6) | 95.3 (17.1) | 102.9 (27.0) | 110.0 (20.2) | U NOBI | - | $p < 0.001$ |
| MVC relative to body mass (Nm/kg) | 2.16 (0.38) | 2.26 (0.45) | 2.09 (0.34) | 1.67 (0.30) | 1.96 (0.67) | 1.63 (0.28) | 1.44 (0.35) | 1.33 (0.37) | LN OBI | LOB | $p < 0.001$ |
| Net PF torque (Nm) | | | | | | | | | | | |
| FF MVC (F | 134.9 (21.4) | 142.1 (25.7) | 161.5 (33.3) | 181.4 (36.6) | 105.6 (28.1) | 109.6 (17.4) | 122.6 (26.9) | 134.7 (21.6) | U NOBI | - | $p < 0.001$ |
| MVC relative to body mass (Nm/kg) | 2.36 (0.36) | 2.48 (0.43) | 2.27 (0.27) | 1.90 (0.30) | 2.03 (0.68) | 1.99 (0.56) | 1.71 (0.35) | 1.52 (0.39) | U NOBI | NOB | $p < 0.001$ |
| DF MVC (F /Nm) | 22.8 (7.2) | 23.5 (5.0) | 27.0 (7.2) | 24.9 (7.0) | 28.0 (4.1) | 19.4 (3.9) | 22.6 (5.6) | 28.4 (6.7) | - | U N OBI | $p = 0.021$ |
| Activation (%) | 95.0 (5.0) | 95.9 (7.8) | 94.1 (6.3) | 87.9 (10.4) | 98.1 (12.1) | 86.4 (10.6) | 82.2 (13.7) | 84.0 (15.2) | - | - | $p < 0.001$ |
| Co-contraction (%) | 15.7 (7.2) | 17.2 (8.5) | 15.2 (5.8) | 16.1 (5.8) | 15.9 (10.6) | 15.1 (8.8) | 12.9 (7.9) | 12.8 (6.8) | - | - | $p = 0.042$ |
| Leg lean mass (kg) | 8.99 (0.79) | 9.30 (0.97) | 7.33 (1.05) | 8.38 (1.35) | 5.37 (0.12) | 5.26 (0.52) | 5.81 (0.64) | 7.21 (1.38) | LN OBI | LN OB | $p < 0.001$ |

Data are presented as means±SD

*UN* underweight, *N* normal weight, *O* overweight, *OB* obese

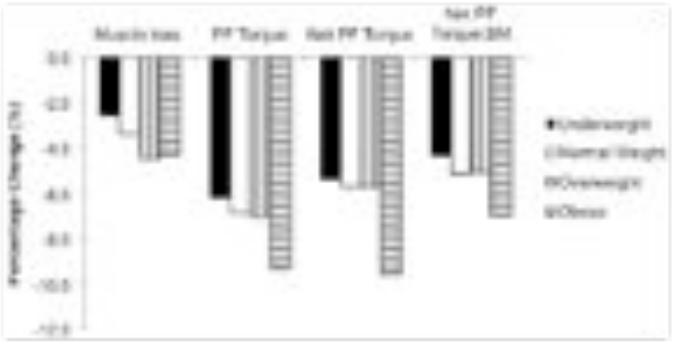


**Fig. 1** Relative change (mean % change per 10 years assuming percentage change is linear) by BMI class (*a*) muscle loss, (*b*) PF torque, (*c*) net PF torque, and (*d*) net PF torque normalised for BM

the effects of ageing and obesity are not necessarily cumulative where absolute joint torque is concerned. Another partially supported hypothesis was also the observation that high adiposity only decreased activation capacity in the young whereas it had no effect in the old individuals. The summation of a negative impact of obesity to ageing in this population was seen in terms of neuromuscular ageing whereby there were marked differences in the slopes of the relationships between adiposity, muscle torque and activation capacity. The above observations culminated in the fact that with torque normalised for body mass and corrected for activation and co-activation, the obesity-induced weakness was true for all pairwise BMI category comparisons in the young. In the old, however, once torque was normalised for body mass and corrected for activation and co-activation, the only significant BMI pairwise difference was between obese vs. normal weight.

## Body composition

The loading effect of chronically high levels of adiposity, body mass and lean mass appears to provide a stimulus to the antigravity muscles of the lower limb similar to resistance training of increased muscle mass (Erskine et al. 2010b) (as shown in Tables 2, 3 and 4). However, whilst 3 weeks of simulated hypergravity (Bosco 1985) would cause adaptations at a neurogenic level, the mean duration of obesity is longitudinal at 13 years (Abdullah et al. 2011), and hence, adaptations to this condition were not expected to be neural led. At the muscle level, low-load, high-volume resistance exercise has been shown to stimulate muscle protein synthesis more than traditional high-load, low-volume resistance exercise (Burd et al. 2010), and our hypothesis was that obesity would have a similar effect, thus leading to increased muscle mass and strength over time.

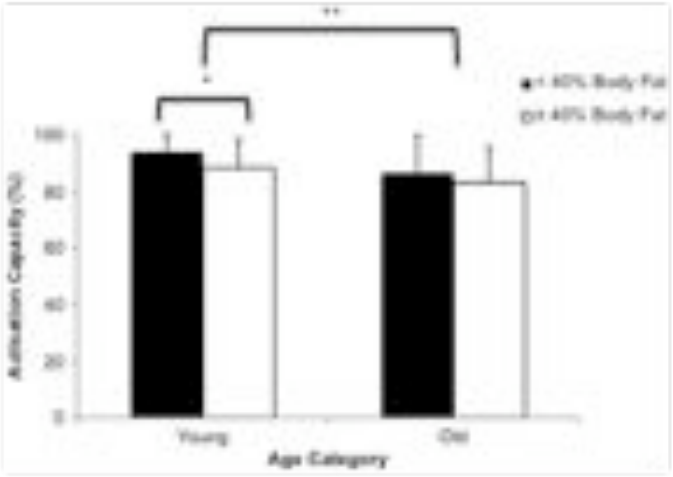


**Fig. 2** Impact of ageing on PF activation capacity in low body fat (<40 %) and high body fat (>40 %) individuals. The threshold set at >40 % body fat as being defined obese is due to previous work in an adult population (Rolland et al. 2004). Data are presented as mean±SD (*$p<0.05$, **$p<0.01$)

Leg lean mass decreased with age in all BMI classifications, but this age-related decrease was exacerbated in the overweight and obese individuals who lost 4.5 and 4.3 %, respectively, compared to 2.6 and 3.4 % in underweight and normal weight individuals every 10 years (data calculated assuming a linear regression, Fig. 1). This blunted effect of loading in the old participants on leg lean mass may possibly be attributed to lower levels of anabolic circulating hormones such as insulin-like growth factor-1 (IGF-I) (Galli et al. 2012) and the increase of catabolic cytokines such as interleukin-6 (IL-6) and tumour necrosis factor α (TNF-α) (Visser et al. 2002; Hotamisligil et al. 1995) seen in older vs. younger individuals.

## Muscle strength

Increased adiposity in the young was previously shown to have a positive effect on the loaded antigravity muscles of the knee extensors in terms of both absolute MVC isometric torque (Hulens et al. 2001; Maffiuletti et al. 2007; Abdelmoula et al. 2012) and isokinetic power (Maffiuletti et al. 2007; Hulens et al. 2002), but not when torque was normalised to body mass, as obese individuals were shown to be weaker (Maffiuletti et al. 2007). Our current results in the plantar flexors mirror these observations.

In the old individuals, there was also a trend of increasing absolute torque with BMI classification, yet there were no significant differences in MVC torque normalised to body mass between BMI classifications (Table 4), except, where the old obese were significantly weaker compared to normal weight individuals. This finding partly supports those of Rolland et al. (2004), who reported greater absolute knee extensor strength in the obese. Additionally, the old obese individuals lost more MVC torque compared to the losses in leg lean mass (27 vs. 13 %); this is comparable to research by Delmonico et al. (2009) who attributed losses in strength to lowering in muscle quality. This may possibly be attributed to intrinsic changes in muscle properties such as selected atrophy of type II fibres (Lexell and Taylor 1991) and/or a decrease in muscle fascicle pennation angle (Morse et al. 2005) normally seen in ageing.

Whilst our old females did not meet the criterion for sarcopenia (i.e. appendicular skeletal muscle mass÷height ($m^2$)÷mean±SD 9.6±1.5 $kg/m^2$ in this group vs. ≤5.67 $kg/m^2$ criterion; Newman et al. 2003), they displayed typical ageing-related characteristics of significantly lower isometric MVC torque (−25 %, $p<0.001$), agonist activation (92.1 vs. 84.5 %, $p<0.05$) and leg lean mass (7.03 vs. 5.92 kg, $p<0.001$). Ageing-associated α-motor neuron degeneration and denervation (Brown 1972), higher levels of IL-6 and TNF-α (Visser et al. 2002) may explain some of these effects. Assuming the loss of joint torque is linear, PF MVC torque appeared to be exacerbated in obese individuals who would lose 9.6 % of maximal torque compared to 5.4, 5.7 and 5.7 % in underweight, normal weight and overweight individuals every 10 years (data calculated assuming a linear regression, Fig. 1).

The effect of adiposity per se on muscle strength suggested a positive association with absolute PF MVC in both young and old groups (Table 2). Yet, when factoring total adiposity in a multiple linear regression, lean muscle mass was the only significant predictor of PF MVC torque. Since adiposity is not a contractile tissue, the apparent positive association with muscle strength can only be due to high adiposity being associated with greater muscle mass in our population.

Previous research on obesity in ageing and muscle performance mainly selected the loaded knee extensors as an indicator of lower limb contractile capacity. The work presented in this paper is novel in that (a) it utilised the plantar flexors, a muscle group which has a key functional relevance to older persons due to its documented contribution in the maintenance of postural balance (Onambele et al. 2006a), so much so that approximately 80 % of the variance in postural balance can be attributed to the functional characteristics of this muscle group, and (b) obesity also has been documented as having a detrimental impact on postural balance (Maffiuletti et al. 2005).

## Co-contraction

To our knowledge, this is the first study to describe antagonist muscle co-contraction in an obese population. Our study reports, at face value, a protective effect of obesity during ageing since old obese individuals had significantly lower co-contraction than their younger counterparts (16.1 vs. 13.8 %, $p<0.042$), thereby potentially contributing to higher agonistic forces and hence better control of joint motion. This was an unexpected finding as ageing is associated with increased co-contraction in the hamstrings (Macaluso et al. 2002) and the triceps surae (Onambele et al. 2006a). However, since joint stabilisation through co-contraction is a

strategy used when muscle weakness is present (Hortobagyi and DeVita 2000), the old obese are in fact doubly disadvantaged through being both weaker and less able to co-contract their antagonist muscles compared to age-matched normal weight counterparts, potentially leading to increased risk of joint pathologies (Felson 1995).

### Voluntary muscle activation

Previous work examining muscle strength differences between obese and non-obese individuals did not account for agonist activation and antagonist co-contraction, thus underestimating the true contractile torque potential, even when such work normalised MVC torque to muscle mass (Abdelmoula et al. 2012; Maffiuletti et al. 2007). Our study demonstrates that high levels of adiposity, with the threshold set at ≥40 % body fat (Rolland et al. 2009), have a significantly negative impact on agonist muscle activation in Y (88 vs. 94 %) but not O (83 vs. 87 %) individuals. The Y data supports the Blimkie et al. (1990) study that reported the agonist activation capacity of obese adolescents aged 15–18 years old to be significantly lower than that of non-obese counterparts (85 vs. 95 %). It would therefore appear that unlike simulated hypergravity which enhances muscle activation (Bosco et al. 1986), obesity-mediated chronic overloading has either no (as seen in the O) or a negative (as seen in the Y) impact on voluntary muscle activation. This would tend to refute the suggestion by Bosco et al. (1986) of a neurogenic mechanism for increased muscle power in the obese. We would argue therefore that any strengthening effect of obesity would be through muscular factors such as increased protein synthesis rate (Burd et al. 2010), which is evidenced through the phenotypic expression of greater absolute lean mass in this population.

A limitation of the present study, as with previous work (Blimkie et al. 1990; Maffiuletti et al. 2007; Abdelmoula et al. 2012), is the failure to quantify anatomical cross-sectional area and using instead leg lean mass and estimations of fat-free mass and thigh muscle mass as an indication of agonist muscle size. Not accounting for muscle fascicle pennation angle (and hence sarcomeres in parallel) and fascicle length (and hence sarcomeres in series) limits the ability to explain differences in force and power. Future work should determine a more accurate index of muscle size by measuring the physiological cross-sectional area (muscle volume/fascicle length) (Fukunaga et al. 1996).

## Conclusion

The present study demonstrates that antigravity muscles adapt to chronically different levels of adiposity in both young and elderly individuals. Interestingly, the magnitude of the effect of obesity in terms of both absolute MVC joint torque and torque normalised to leg lean mass appears to be blunted in the older group. Also notable, the rate of ageing (i.e. the slope of deleterious changes in neuromuscular properties) in the BMI subcategories is most dramatic for the high-adiposity groups.

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**Conflict of interest** We do declare that we have no conflict of interest.



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# The impact of obesity on skeletal muscle architecture in untrained young vs. old women

D. J. Tomlinson, R. M. Erskine,* K. Winwood, C. I. Morse and G. L. Onambélé

*Department of Exercise and Sport Science, Institute for Performance Research, Manchester Metropolitan University, Crewe, UK*

## Abstract

It is unknown whether loading of the lower limbs through additional storage of fat mass as evident in obesity would promote muscular adaptations similar to those seen with resistance exercise. It is also unclear whether ageing modulates any such adjustments. This study aimed to examine the relationships between adiposity, ageing and skeletal muscle size and architecture. A total of 100 untrained healthy women were categorised by age into young (Y) (mean ± SD: 26.7 ± 9.4 years) vs. old (O) (65.1 ± 7.2 years) and body mass index (BMI) classification (underweight, normal weight, overweight and obese). Participants were assessed for body fat using dual energy x-ray absorptiometry, and for gastrocnemius medialis (GM) muscle architecture (skeletal muscle fascicle pennation angle and length) and size [GM muscle volume and physiological cross-sectional area (PCSA)] using B-mode ultrasonography. GM fascicle pennation angle (PPA) in the obese Y females was 25% greater than underweight ($P = 0.001$) and 25% greater than normal weight ($P = 0.001$) individuals, while O females had 32 and 22% greater PPA than their underweight ($P = 0.008$) and normal weight ($P = 0.003$) counterparts. Furthermore, PPA correlated with body mass in both Y and O females (Y $r = 0.303$; $P < 0.001$; O $r = 0.223$; $P = 0.001$), yet no age-related differences in the slope or $r$-values were observed ($P > 0.05$). Both GM muscle volume ($P = 0.003$) and PCSA ($P = 0.004$) exhibited significant age × BMI interactions. In addition, muscle volume and PCSA correlated with BMI, body mass and fat mass. Interestingly, ageing reduced both the degree of association in these correlations ($P < 0.05$) and the slope of the regressions ($P < 0.05$). Our findings partly support our hypotheses in that obesity-associated changes in GM PCSA and volume differed between the young and old. The younger GM muscle adapted to the loading induced by high levels of body mass, adiposity and BMI by increasing its volume and increasing its pennation angle, ultimately enabling it to produce higher maximum torque. Such an adaptation to increased loading did not occur in the older GM muscle. Nonetheless, the older GM muscle PPA increased to a similar extent to that seen in young GM muscle, an effect which partly explains the relatively enhanced absolute maximum torque observed in obese older females.

**Key words**: adiposity; ageing; muscle volume; obesity; physiological cross-sectional area.

## Introduction

Obesity in both young and old individuals has been shown to induce a loading effect on skeletal muscles of the lower limbs (Lafortuna et al. 2013), increasing absolute maximal voluntary contraction (MVC) torque in obese compared with both normal and underweight individuals (Rolland et al. 2004; Maffiuletti et al. 2007; Tomlinson et al., 2014). A plausible explanation for higher absolute strength may be attributed to greater fat-free mass (FFM) seen in obese individuals (Maffiuletti et al. 2007). However, no previous study has quantified physiological cross-sectional area (PCSA) or muscle architectural component differences in the pennate anti-gravity muscles of the lower limb in obese and non-obese individuals. This is key, as PCSA, more than FFM, allows for the identification of intrinsic muscle quality (strength per unit of PCSA) differences, where fascicle length and pennation angle (i.e. architecture) effects are highlighted.

The potential impact of using muscle-specific PCSA measures rather than whole limb estimates of FFM may explain the apparent discrepancy within the literature on the currently reported impact of obesity on muscle mass. Blimkie et al. (1990) reported no difference between obese

Correspondence
*Dave Tomlinson, Department of Exercise and Sport Science, Institute for Performance Research, Manchester Metropolitan University, Crewe Green Road, Crewe CW1 5DU, UK. T: + 44 (0)161 2476386; E: g.pearson@mmu.ac.uk*

*†Present address: Research Institute for Sport and Exercise Sciences Liverpool John Moores University Liverpool, L3 3AF, UK*

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and non-obese adolescents in quadriceps anatomical cross-sectional area (ACSA) using CT. This was reiterated by Abdelmoula et al. (2012) from estimated thigh muscle mass using DEXA. In contrast, Maffiuletti et al. (2007) reported 18% greater fat-free mass in obese adults using bioelectrical impedance, whereas previous authors (Rolland et al. 2004) reported similarly increased leg muscle mass using DEXA in an elderly obese population. PCSA is directly proportional to the maximum force generated by skeletal muscle (Lieber & Friden, 2000; Maganaris et al. 2001). Therefore using PCSA as a measure of muscle size would improve data comparison accuracy over ACSA and/or estimations of lean mass utilised in previous studies, as highlighted in the paragraph above. Indeed, ACSA and lean mass estimates would potentially underestimate PCSA (volume/fascicle length; Alexander & Vernon, 1975), thereby leading to an inaccurate estimation of intrinsic skeletal muscle quality.

Ageing, and specifically sarcopenia, is characterised by reduced muscle PCSA, and fascicle pennation angle and length (Morse et al. 2005*a*). Slowing down the effects of ageing on skeletal muscle is achievable through resistance training and sustained hypergravity (Brown et al. 1990; Ferri et al. 2003; Reeves et al. 2004*b*; Klentrou et al. 2007; Morse et al. 2007). In contrast to the benefits of resistance exercise or simulated hypergravity, excess adiposity does not appear to be enough of a loading stimulus to mitigate the detrimental functional consequences of obesity in the elderly (e.g. difficulties in walking, climbing stairs and rising from a chair; Rolland et al. 2009). Additionally, a condition that has been shown to exacerbate functional limitations, known as 'sarcopenic obesity', is characterised by the age-related loss of muscle mass and strength plus greater intramuscular fat infiltration (Baumgartner, 2000). These increases in fat infiltration coupled with sarcopenia in the elderly are reported to lead to higher levels of pro-inflammatory cytokines associated with muscle catabolism (Schrager et al. 2007), and hence potentially greater prevalence of decreased skeletal muscle mass.

To date, no study has examined the combined effect of sarcopenia and obesity in the elderly, on muscle architecture. This is a patently important area of study, as a further loss of sarcomeres in parallel in the obese, would detrimentally affect maximal force production, thus highlighting the need to target this population for specific counter-measures.

The primary aim of the present study was to examine the degree of any association between BMI (or adiposity per se, i.e. irrespective of BMI status) and muscle architecture (fascicle length and pennation angle), as well as PCSA. A second aim was to determine whether the effects of ageing and adiposity (i.e. continued adiposity from younger to older age) were additive on these variables. It was hypothesised that: (i) muscle PCSA in both obese young and old would be greater than in lean, normal weight and overweight individuals; (ii) muscle fascicle pennation angle and length in obese young and old would be greater than in lean, normal weight and overweight individuals; and (iii) the slope of the relationship between adiposity, BMI, or body mass and PCSA, muscle volume, or architecture, would be lower in the older individuals than in their younger counterparts, denoting a faster rate of changes with increased ageing.

## Method

### Participants

A total of 100 untrained females volunteered to take part in this study and were categorised by age into either Young (Y) 18-49 years old or Old (O) 50-80 years old (Table 1). Participants were then sub-categorised into four body mass index (BMI) classifications [BMI (kg) stature$^{-2}$ (m)] into Underweight (BMI < 20), Normal (BMI 20-24.9), Overweight (BMI 25-29.9) and Obese (BMI > 30). The principal exclusion criteria were issues with lower limb muscle/joints affecting mobility or ability to exert maximum torque. It should be noted here that use of non-steroidal anti-inflammatory drugs was also an exclusion criterion. In addition, although three study participants had controlled type II diabetes mellitus, they did not in fact display any characteristics of peripheral neuropathy, such as motor dysfunction and weakness. Physical activity status was screened by questionnaire and participants were excluded if they self-reported as habitually undertaking structured exercise for more than 3 h per week.

Participants gave written informed consent to this study prior to undertaking any assessment, which was approved by the local university Ethics committee.

### Body composition measure

A dual energy x-ray absorptiometry (DEXA) scanner (Hologic Discovery; Vertec Scientific Ltd, UK) was used to ascertain 12 h fasted whole body composition. Participants lay in a supine position, avoiding any contact between the trunk and the appendicular mass during a 7 min scanning procedure (whole body procedure, EF 8.4 v3r). Appendicular skeletal muscle mass (ASM) was estimated from the DEXA as the total muscle mass of both the upper and lower limbs. The appendicular skeletal muscle mass index was then calculated using the following calculation: ASM height$^{-2}$ (kg.m$^{-2}$).

### Muscle architecture

Muscle architecture of the gastrocnemius medialis (GM) was measured using B-mode ultrasonography (iU/i Harmonic, Esaote Biomedica, Genoa, Italy) both at rest and during a graded maximal MVC over 6 s. Participants were seated in an isokinetic dynamometer (Cybex Norm, Cybex International, New York, NY, USA) with their hip at an 85° angle, dominant leg extended, and their foot secured to the footplate of the dynamometer. Participants were strapped into the dynamometer using non-extending straps at the hip, distal thigh and chest to reduce extraneous movements.

Resting fascicle pennation angle (PA) and fascicle length (*L*f) were measured with the probe (7.5 MHz linear array probe, 38 mm wide) positioned at 50% of the GM muscle length, at mid muscle belly in the sagittal plane as shown in Fig. 1. Participants were then

**Table 1** Descriptive variables for BMI classifications in both young and old age classifications. Data are presented as mean ± SD.

| Young (18–40) | Underweight (*n* = 13) | Normal (*n* = 53) | Overweight (*n* = 9) | Obese (*n* = 17) | BMI effect | Ageing effect |
|---|---|---|---|---|---|---|
| Age (years) | 23.8 ± 6.7 | 23.2 ± 7.9 | 23.6 ± 8.0 | 30.9 ± 10.7 | $P = 0.002$ | $P = 0.001$ |
| BMI (kg m$^{-2}$) | 18.8 ± 0.9 | 21.6 ± 1.1 | 26.1 ± 2.4 | 35.2 ± 4.4 | $P < 0.001$ | $P = 0.625$ |
| Body fat % | 26.5 ± 3.9 | 30.4 ± 3.5 | 38.7 ± 5.9 | 45.3 ± 3.9 | $P < 0.001$ | $P = 0.002$ |
| Fat Mass (kg) | 13.7 ± 2.2 | 17.2 ± 2.7 | 28.5 ± 6.8 | 43.2 ± 7.3 | $P < 0.001$ | $P = 0.166$ |
| Appendicular skeletal muscle mass (ASM) (kg) | 15.8 ± 1.8 | 16.1 ± 2.6 | 18.7 ± 2.7 | 21.3 ± 3.5 | $P < 0.001$ | $P = 0.001$ |
| ASM/height$^2$ (kg m$^{-2}$) | 9.4 ± 0.9 | 9.8 ± 1.1 | 11.5 ± 1.4 | 12.8 ± 1.8 | $P < 0.001$ | $P = 0.001$ |
| **Old (50–80)** | **Underweight (*n* = 4)** | **Normal (*n* = 15)** | **Overweight (*n* = 18)** | **Obese (*n* = 11)** | **BMI Effect** | **Ageing Effect** |
| Age (years) | 63.8 ± 5.7 | 63.5 ± 7.7 | 68.2 ± 4.8 | 62.5 ± 9.0 | $P = 0.183$ | $P = 0.001$ |
| BMI (kg m$^{-2}$) | 19.1 ± 0.8 | 22.2 ± 1.8 | 27.3 ± 1.2 | 34.1 ± 5.7 | $P < 0.001$ | $P = 0.625$ |
| Body fat % | 26.5 ± 2.1 | 36.0 ± 3.6 | 42.9 ± 3.3 | 46.1 ± 5.0 | $P < 0.001$ | $P = 0.002$ |
| Fat mass (kg) | 12.5 ± 2.0 | 19.9 ± 2.9 | 29.8 ± 3.4 | 40.9 ± 11.3 | $P < 0.001$ | $P = 0.166$ |
| ASM (kg) | 14.4 ± 1.2 | 13.9 ± 1.2 | 15.2 ± 1.6 | 16.5 ± 3.7 | $P < 0.001$ | $P = 0.001$ |
| ASM/height$^2$ (kg m$^{-2}$) | 9.0 ± 0.7 | 8.7 ± 0.6 | 9.4 ± 0.9 | 11.4 ± 1.8 | $P < 0.001$ | $P = 0.001$ |

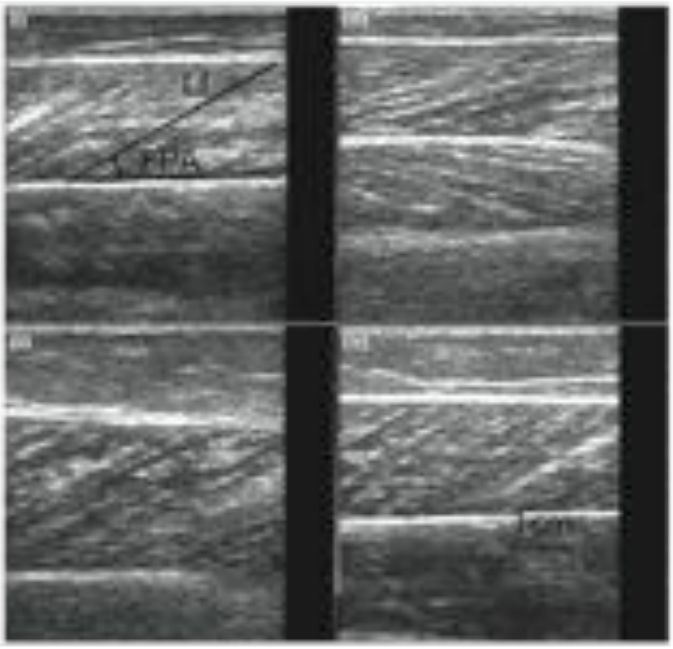


**Fig. 1** Representative sagittal plane sonograms of the gastrocnemius medialis at 50% of its muscle length in a (i) young normal weight female, (ii) young obese female, (iii) old normal weight female and (iv) old obese female. FPA = fascicle pennation angle; LF = fascicle length.

asked to perform a ramped MVC over 4 s, where the change in both FPA and LF were recorded on the capturing software (MotoMo version no. Version 6, Adobe Systems Software, Ireland). Both resting and maximal images (the latter synchronised with torque outputs using a square wave signal generator) were extrapolated from the capturing software and analysed using ImageJ (1.45 s, National Institutes of Health, Bethesda, MD, USA). Three clearly visible fascicles within the capturing window, defined from the deep to the superficial aponeurosis, were analysed and the mean values of LF and FPA were recorded. FPA was defined as the angle of the fascicular path from the superficial to the deep aponeuroses (datum line) of the GM muscle. Linear extrapolation was used on fascicles that extended beyond the edge of the screen. Extrapolation was only undertaken if 60% of the chosen fascicle was visible within

the scanning window in line with previous methodology examining muscle architecture of the GM in both a young and old population (Morse et al. 2005a).

## Muscle volume

GM muscle volume was calculated using the truncated cone method through the construction of several ACSAs taken at discrete muscle sites (25, 50 and 75% of GM length) using B-mode ultrasonography (AU5 Harmonic, Esaote Biomedical). Participants lay in the prone position with their ankle positioned in neutral (90° angle, referred here as 0°). B-mode ultrasonography was then used to ascertain the proximal insertion (0% of total length) and distal insertion (100% of total length) of the GM, where discrete muscle sites (0, 25, 50, 75 and 100% of length) were marked from the medial to lateral border of the GM. Thin strips (2 mm) of micropore tape (3M, Bracknell, UK) were placed axially 3–4 cm apart, transversally along the nominated muscle lengths (see Fig. 2). The micropore tape was utilised as an echo-absorptive marker in the schematic reconstruction of ACSAs using photo editing software (corel photo-paint, Version 10). During recording of the ACSA, the ultrasound probe (7.5 MHz linear array probe, 38 mm wide) was held perpendicular to the GM on its medial border and moved along a designated marked pathway to its lateral border to ensure the probe was kept perpendicular to the GM during the whole scanning procedure. The probe was moved steadily across the leg with a constant light pressure to avoid compression of the dermal surface (and hence the muscle) during scanning. This procedure was repeated twice at each muscle site for reliability purposes.

Using the 'shadows' cast by the micropore tape as well as anatomical markers, individual transverse frames were extracted offline from each ultrasound recording to reconstruct GM ACSAs at each of the three muscle lengths of interest (Fig. 3) (Reeves et al. 2004a). Following this manual reconstruction of the three ACSAs at 25, 50 and 75% of muscle length, the areas of the complete transverse ACSAs were undertaken using the analysis software imagej (1.45s; National Institutes of Health). To calculate the total muscle volume, an area of 0.5 $cm^2$ was used as a standard measure for 0 and 100% positions along the GM muscle length. Muscle volume was then calculated using the truncated cone method (there were four cones in total):

$$\text{Cone volume} = (1/3)\,3 \times h) \times \pi \times (R1^2 + R1) + (R2^2 + R2)$$

where R1 = radius of the base ACSA; R2 = radius of the top ACSA; h = distance between segments; π = (ΔCSA/Δ), where π = 3.142.

PCSA was then subsequently calculated using the ratio between GM LT to muscle volume (PCSA = GM muscle volume ($cm^3$)/LT (cm)).

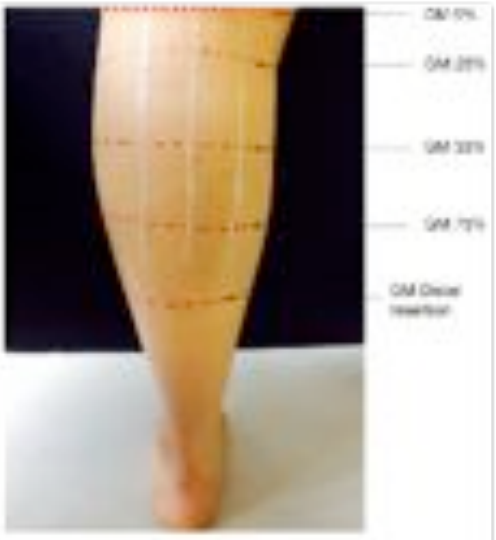


**Fig. 2** Schematic detailing the anatomical markings at the discrete muscle lengths along the gastrocnemius medialis (GM) muscle length (25, 50 and 75%) and placement of the micropore tape. The GM insertion distal constitutes the 100% muscle length and the GM proximal insertion, the 0% length.

## Reliability

The reliability in the measurement of both muscle architectural characteristics (muscle fascicle pennation angle and length) and GM ACSA was measured in 10 participants (F = 5; O = 5; BMI range = 17.6–36.7 $Kg/m^2$) on two different days (separated by at least 48 hrs) by the same investigator.

The intra class coefficients (absolute agreement) for all the measurements were high and significant for all of the assessment techniques (muscle fascicle pennation angle rest = 0.997, muscle fascicle pennation angle max = 0.997, muscle fascicle length rest = 0.998, muscle fascicle length max 0.993, GM ACSA 25% length = 0.998, GM ACSA 50% length = 0.999, GM ACSA 75% length = 0.998). It is notable that the measurements of the ACSAs used in the construction of muscle volume are reliable and demonstrate strong agreement with MRI-obtained values (Reeves et al. 2004a).

## Statistical analyses

Statistical analyses were carried out using spss (Version 19, SPSS Inc., Chicago, IL, USA). To determine parametricity, Kolmogorov-Smir-

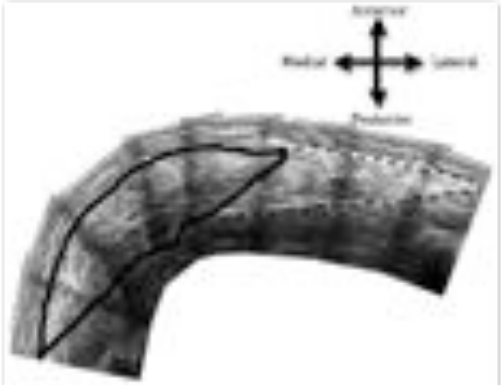


**Fig. 3** Reconstructed axial plane scan of the gastrocnemius medialis (GM) anatomical cross-sectional area at 50% of muscle length using ultrasonography.

nov (Y participants) or Shapiro–Wilk (O participants) (normal distribution) and Levene's tests (homogeneity of variance) were utilised. If parametric assumptions were met (PFA, LF, LTmuscle length, GM muscle volume and GM PCSA), a factorial 2 × 4 ANCOVA (Age × BMI) was utilised with post hoc Bonferroni correction for pairwise comparisons. Where parametric assumptions were breached (age, BMI, fat mass, ASM and ASMI height$^{-2}$), a Mann–Whitney or Kruskal–Wallis test was utilised as appropriate. Pearson correlations described the relationships between measures of muscle architecture, with body mass, fat mass, total lean mass, body fat % and BMI. Comparison of the regression coefficients and slopes were conducted using z-transformations and the Student's t-statistic. It should be noted that some participants did not complete all tests due to faults during data capture, hence the data on regressions utilises fewer samples than the complete cohort of 188 participants (see Results, Table 3). Data are reported as mean ± SD and statistical significance was accepted when $P \leq 0.05$. Study power (β) and effect size ($\eta_p^2$) are also reported.

## Results

### Body composition

Table 1 displays descriptive values for age, BMI, body fat%, ASM and ASM height$^{-2}$ (m) for Y and O females categorised by BMI.

### Muscle pennation angle

Muscle PPA at rest revealed a main effect of age ($P = 0.036$; $\eta_p^2 = 0.047$; $\beta = 0.556$) and BMI ($P < 0.001$; $\eta_p^2 = 0.337$; $\beta = 1.000$), but no significant age × BMI interaction ($P = 0.190$; $\eta_p^2 = 0.053$; $\beta = 0.413$). However, Y obese had 16 and 24% larger muscle PPA at rest than Y underweight ($P = 0.020$) and Y normal weight ($P < 0.001$) individuals, whereas O obese had 38% and 20% larger muscle PPA at rest than Y underweight ($P = 0.001$) and Y normal weight ($P = 0.005$) individuals (Table 2).

Muscle PPA during a maximum isometric contraction revealed a main effect of age ($P = 0.005$; $\eta_p^2 = 0.063$; $\beta = 0.813$) and BMI ($P < 0.001$; $\eta_p^2 = 0.362$; $\beta = 1.000$), but no significant age × BMI interaction ($P = 0.883$; $\eta_p^2 = 0.003$; $\beta = 0.090$). However, Y obese had 25% larger muscle PPA during a maximum isometric contraction than Y underweight ($P = 0.001$) and Y normal weight ($P = 0.001$) individuals, whereas O obese had 32% and 22% larger muscle PPA during a maximum isometric contraction than Y underweight ($P = 0.008$) and Y normal weight ($P = 0.003$) individuals (Table 2).

### Muscle fascicle length

Muscle LF at rest revealed no significant effects of age ($P = 0.537$; $\eta_p^2 = 0.004$; $\beta = 0.094$), BMI ($P = 0.789$; $\eta_p^2 = 0.011$; $\beta = 0.116$) or age × BMI interaction ($P = 0.227$; $\eta_p^2 = 0.041$; $\beta = 0.339$) (Table 2).

Similarly, muscle LF during a maximum isometric contraction revealed no significant effects of age ($P = 0.063$; $\eta_p^2 = 0.037$; $\beta = 0.461$), BMI ($P = 0.376$; $\eta_p^2 = 0.021$; $\beta = 0.185$) or age × BMI interaction ($P = 0.653$; $\eta_p^2 = 0.017$; $\beta = 0.158$) (Table 2).

### Muscle anatomical cross-sectional area

GM ACSA at 25% of muscle length revealed a main effect of BMI ($P < 0.001$; $\eta_p^2 = 0.217$; $\beta = 0.988$), an age effect

**Table 2** Displays GM skeletal muscle characteristics (GM muscle architecture, GM anatomical cross-sectional area, GM muscle volume and GM physiological cross-sectional area) in both young and old BMI classifications. Data are presented as mean ± SD.

| | Young | | | | Old | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Underweight ($n = 10$) | Normal ($n = 10$) | Overweight ($n = 9$) | Obese ($n = 17$) | Underweight ($n = 8$) | Normal ($n = 15$) | Overweight ($n = 18$) | Obese ($n = 11$) | Young BMI effect | Old BMI effect | Ageing effect |
| GM muscle architecture | | | | | | | | | | | |
| PPA (°) – Rest | 18.8 ± 2.3 | 17.9 ± 2.9 | 21.2 ± 2.9 | 21.4 ± 2.3 | 17.3 ± 1.9 | 17.9 ± 2.2 | 19.8 ± 2.8 | 21.4 ± 2.7 | U NOSt | U NOSt | $P = 0.004$ |
| PPA (°) – Max | 28.8 ± 5.8 | 28.3 ± 4.9 | 31.8 ± 4.8 | 35.8 ± 4.8 | 24.9 ± 3.9 | 26.8 ± 3.2 | 29.3 ± 4.6 | 32.8 ± 3.6 | U NOSt | U NOSt | $P = 0.005$ |
| LF (cm) – Rest | 5.2 ± 0.6 | 5.3 ± 0.6 | 5.5 ± 0.8 | 5.7 ± 0.7 | 5.1 ± 0.6 | 5.6 ± 0.7 | 5.4 ± 1.0 | 5.6 ± 0.7 | - | - | $P = 0.027$ |
| LF (cm) – Max | 3.7 ± 0.7 | 3.6 ± 0.4 | 3.9 ± 0.6 | 3.7 ± 0.6 | 4.1 ± 0.4 | 4.0 ± 0.7 | 3.8 ± 0.6 | 3.9 ± 0.5 | - | - | $P = 0.063$ |
| GM muscle size | | | | | | | | | | | |
| GM 25% ACSA (cm$^2$) | 8.6 ± 2.3 | 8.7 ± 2.1 | 13.8 ± 3.9 | 14.9 ± 2.8 | 11.2 ± 2.0 | 9.7 ± 2.0 | 15.2 ± 2.1 | 15.7 ± 2.5 | U NOSt | - | $P = 0.020$ |
| GM 50% ACSA (cm$^2$) | 12.1 ± 1.9 | 10.1 ± 2.0 | 17.1 ± 4.2 | 21.3 ± 4.7 | 12.4 ± 1.4 | 13.7 ± 2.1 | 14.8 ± 3.4 | 16.9 ± 4.6 | U NOSt | - | $P = 0.110$ |
| GM 75% ACSA (cm$^2$) | 8.1 ± 1.8 | 8.9 ± 1.9 | 11.2 ± 2.1 | 14.8 ± 2.9 | 10.8 ± 2.4 | 8.5 ± 1.8 | 9.3 ± 2.3 | 10.3 ± 2.4 | U N OSt | UNSt | $P < 0.001$ |
| GM Muscle Volume (cm$^3$) | 189.4 ± 36.7 | 185.0 ± 37.3 | 257.5 ± 83.9 | 319.4 ± 56.9 | 182.4 ± 27.1 | 194.0 ± 40.1 | 288.1 ± 35.4 | 226.3 ± 48.7 | U NOSt | - | $P = 0.010$ |
| GM PCSA (cm$^2$) | 59.8 ± 11.9 | 52.1 ± 12.8 | 67.8 ± 17.8 | 88.3 ± 16.3 | 66.3 ± 8.1 | 68.3 ± 11.2 | 51.3 ± 10.3 | 84.3 ± 13.5 | U N OSt | - | $P < 0.001$ |

ACSA, anatomical cross-sectional area; PPA, fascicle pennation angle; LF, fascicle length; N, normal weight; O, overweight; Ob, obese; PCSA, physiological cross-sectional area; U, underweight.

($P = 0.020$; $\eta p^2 = 0.061$; $\beta = 0.650$), and an age × BMI interaction ($P = 0.001$; $\eta p^2 = 0.179$; $\beta = 0.961$). This translated to Y obese having 68% and 61% greater GM ACSA than Y underweight ($P < 0.001$) and Y normal weight ($P < 0.001$) individuals, whereas O obese individuals did not have significantly greater ACSA than their underweight, normal weight and overweight counterparts ($P > 0.05$) at that site (Table 2).

GM ACSA at 50% of muscle length revealed a main effect of BMI ($P < 0.001$; $\eta p^2 = 0.365$; $\beta = 1.000$), no significant age effect ($P = 0.110$; $\eta p^2 = 0.029$; $\beta = 0.359$) and no age × BMI interaction ($P = 0.059$; $\eta p^2 = 0.081$; $\beta = 0.617$). This translated to Y obese having 76% and 62% greater GM ACSA than Y underweight ($P < 0.001$) and Y normal weight ($P < 0.001$) individuals, whereas O obese individuals did not have significantly greater ACSA than their underweight, normal weight and overweight counterparts ($P > 0.05$) (Table 2).

GM ACSA at 75% of muscle length revealed a main effect of BMI ($P < 0.001$; $\eta p^2 = 0.371$; $\beta = 1.000$) as well as an age effect ($P < 0.001$; $\eta p^2 = 0.144$; $\beta = 0.968$), but no age × BMI interaction ($P = 0.062$; $\eta p^2 = 0.080$; $\beta = 0.609$). More specifically, Y obese had 74%, 58% and 24% greater GM ACSA than Y underweight ($P < 0.001$), Y normal weight ($P < 0.001$) and Y overweight ($P = 0.048$) individuals, whereas O obese individuals only had 2% lower ACSA than their underweight counterparts ($P = 0.046$) (Table 2).

### Muscle volume

GM muscle volume data revealed a main effect of age ($P = 0.010$; $\eta p^2 = 0.074$; $\beta = 0.745$), BMI ($P < 0.001$; $\eta p^2 = 0.354$; $\beta = 1.000$) and an age × BMI interaction ($P = 0.003$; $\eta p^2 = 0.145$; $\beta = 0.897$). Thus, Y obese had 71% and 73% greater GM muscle volume than Y underweight ($P < 0.001$) and Y normal weight ($P < 0.001$) individuals, whereas O obese individuals did not have significantly greater GM muscle volume than their underweight, normal weight and overweight counterparts ($P > 0.05$) (Table 2).

### Muscle physiological cross-sectional area

GM PCSA revealed a main effect of age ($P < 0.001$; $\eta p^2 = 0.185$; $\beta = 0.952$), BMI ($P < 0.001$; $\eta p^2 = 0.371$; $\beta = 1.000$) and an age × BMI interaction ($P = 0.004$; $\eta p^2 = 0.141$; $\beta = 0.882$). Specifically, Y obese had 77%, 70% and 31% larger GM PCSA than Y underweight ($P < 0.001$), Y normal weight ($P < 0.001$) and Y overweight ($P = 0.017$) individuals, whilst O obese individuals did not have significantly larger GM PCSA than their underweight, normal weight and overweight counterparts ($P > 0.05$) (Table 2).

### Associations between muscle architecture and body composition according to age

Muscle FPA during a maximum isometric contraction and FMI were correlated in both the Y ($P < 0.001$; $r^2 = 0.303$) and O ($P < 0.001$; $r^2 = 0.233$) age groups, with similar slopes in the two age groups (Fig. 4A). Similar correlations were observed during resting conditions between skeletal muscle FPA and FMI in both Y ($P < 0.001$; $r^2 = 0.233$) and O ($P = 0.001$; $r^2 = 0.225$) groups, with similar slopes for the two age groups (Table 3).

There were strong positive associations between GM muscle volume and body mass, fat mass and BMI in both Y and O groups (Table 3). Ageing decreased the strength of the associations, in that both the correlation coefficients and the slopes of the regressions were less strong in the O group ($P < 0.05$, Table 3).

There were strong positive associations between PCSA and body mass, fat mass and BMI in both Y ($P < 0.001$) and O groups ($P = 0.009$) (Table 3, Fig. 4B). Ageing affected both the correlation coefficient in these associations

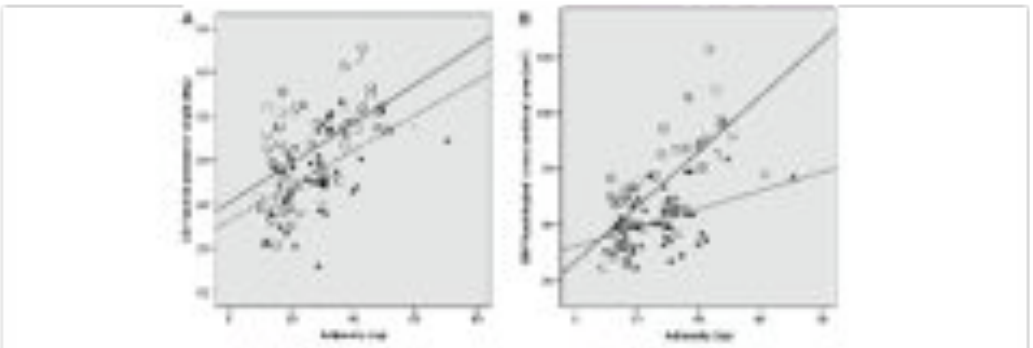

**Fig. 4** Displays the impact of fat mass on gastrocnemius medialis (GM) fascicle pennation angle during maximum isometric contraction and GM physiological cross-sectional area in both young (○) ——— A: $r^2 = 0.303$, $P < 0.001$, B: $r^2 = 0.369$, $P < 0.001$ and old (●) ——— A: $r^2 = 0.233$, $P = 0.001$, B: $r^2 = 0.140$, $P = 0.009$, females.

**Table 3** Pearson correlations, *z* transformation of *r* and Student's *t* statistic between gastrocnemius medialis (GM) muscle volume and physiological cross-sectional area (PCSA) and fascicle pennation angle (FPA) vs. a series of descriptive variables in young and old untrained females.

| | Young | | | Old | | | Correlation co-efficient | Ageing effect |
|---|---|---|---|---|---|---|---|---|
| | *N* | *r* value | slope | *N* | *r* value | Slope | *Z* transformation of *r* | Student's *t* statistic |
| GM muscle volume vs. BM | 50 | 0.82*** | 3.15 | 45 | 0.47*** | 1.19 | 2.39* | 3.15* |
| GM muscle volume vs. FM | 50 | 0.76*** | 4.54 | 45 | 0.40** | 1.52 | 2.37* | 3.90* |
| GM muscle volume vs. BMI | 50 | 0.75*** | 8.23 | 45 | 0.43** | 3.13 | 2.03* | 3.51* |
| GM PCSA vs. BM | 49 | 0.81*** | 0.86 | 45 | 0.45** | 0.32 | 2.45* | 2.61** |
| GM PCSA vs. FM | 49 | 0.75*** | 1.24 | 45 | 0.39** | 0.41 | 2.34* | 3.77** |
| GM PCSA vs. BMI | 49 | 0.72*** | 2.17 | 45 | 0.39** | 0.88 | 2.02* | 3.26** |
| FPA (rest) vs. BM | 51 | 0.50*** | 0.73 | 48 | 0.49** | 0.89 | 0.03 | −6.79** |
| FPA (rest) vs. FM | 51 | 0.42*** | 0.11 | 48 | 0.48** | 0.13 | 0.07 | 0.50 |
| FPA (rest) vs. BMI | 51 | 0.53*** | 0.22 | 48 | 0.52*** | 0.27 | −0.02 | 0.55 |
| FPA (max) vs. BM | 51 | 0.60*** | 0.16 | 48 | 0.52*** | 0.15 | 0.43 | 0.28 |
| FPA (max) vs. FM | 51 | 0.55*** | 0.23 | 48 | 0.47** | 0.28 | 0.36 | 0.35 |
| FPA (max) vs. BMI | 51 | 0.57*** | 0.43 | 48 | 0.50*** | 0.48 | 0.43 | 0.21 |

BM, body mass; BMI, body mass index; FM, fat mass.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. If $Z > 1.96$, $P < 0.05$; $Z > 2.58$, $P < 0.01$, Student's *t* statistic significance if *r* falls outside ± 1.96, $P < 0.05$.

($P < 0.05$) and the slope of the regressions ($P < 0.05$, Table 3).

## Discussion

Our data support the hypothesis that high body mass (and/or high BMI and/or high levels of adiposity (absolute fat mass)) acts as a loading stimulus to the GM muscle, particularly in the young. Indeed, GM muscle PCSA, volume and fascicle pennation angle were significantly higher in young obese women than in their normal weight counterparts. Interestingly, even though GM muscle FPA was found to increase, muscle *Lf* did not change with BMI. This effect, functionally, would translate into a potential for increased force but not increased speed of contraction with obesity.

Irrespective of BMI, there were no significant differences in muscle *Lf* between Y and O individuals. However, as expected, Y individuals had significantly higher GM PCSA, GM muscle volume and muscle FPA compared with O individuals. Interestingly, there were significant differences in the positive association between PCSA and BMI, and between body mass and fat mass, in Y compared with O individuals. This suggests that the loading stimulus of high body mass (and particularly associated with high levels of adiposity) is partially blunted in the O cohort, possibly through higher levels of circulating pro-inflammatory cytokines and/or lower anabolic growth hormones previously associated with ageing and obesity (Schrager et al. 2007).

### Muscle architecture

To our knowledge, this is the first study to compare muscle architecture in non-obese vs. obese human adults. This study confirms previous reports (Narici et al. 2003) that muscle FPA decreases with age (Table 2) but that muscle *Lf* does not change with age or BMI classification (Table 2).

It was found that muscle FPA at rest and during maximum muscle contraction increases with BMI classification in both Y (rest: 15, 23 and 1%; max 25, 25 and 13%) and O (rest 38, 20 and 8%; max 32, 22 and 10%) individuals (for underweight, normal, overweight people, respectively, Table 2). An increase in FPA allows for more sarcomeres to be arranged in parallel, which in humans suggests hypertrophy at the single fibre level (Clark et al. 2011). This in turn enables an increase in MVC torque, as long as an increase in FPA does not exceed 45°, when the resultant force resolved at the tendon becomes negative (Alexander & Vernon, 1975; Degens et al. 2009). This finding is emphasised in Fig. 4A, demonstrating that as fat mass increases, muscle FPA in both Y ($r^2 = 0.303$; $P < 0.001$) and O ($r^2 = 0.223$; $P = 0.001$) individuals increases. Within this association there were no differences in the slope of the regression or comparison of the correlation coefficients between age categories ($P > 0.05$), suggesting that the loading effect of adiposity on muscle FPA is similar in Y and O individuals. These increases in FPA both at rest and during maximal contraction reflect the responses seen in bodybuilders, who chronically load their musculature with weight with the aim of increasing muscle mass, and who have been shown to possess a greater FPA when compared with normal weight controls (Kawakami et al. 1993).

Whether the obesity-mediated beneficial increases in FPA allow more contractile material between the aponeuroses (which is likely to be indicative of fibre hypertrophy as observed in diet-induced obesity in pigs (Clark et al. 2011)), and whether this effect is the same in both Y and O obese

individuals, remains to be confirmed. Alternatively, obesity could cause pseudo-hypertrophy, whereby excessive fat infiltrates the muscle, thus artificially increasing muscle thickness and altering the fascicle pennation angle. Fat infiltration has previously been reported in the skeletal musculature of the elderly (Borkan et al. 1983; Visser et al. 2005; Delmonico et al. 2009) and is linked to a lowering of the intrinsic force-generating capacity of the whole muscle (Morse et al. 2005b).

There were no differences in muscle $L_f$ either between Y and O individuals ($P = 0.963$) or BMI sub-categories ($P = 0.376$). As this was the first study to examine the effect of adiposity on muscle fascicle geometry, there appears to be no research to compare the effect of adiposity on $L_f$. Nevertheless, it is notable that research examining the ageing response on fascicle geometry, reports varying results in the gastrocnemius. For instance, Kubo et al. (2003) reported that neither GM muscle PPA ($r = -0.112$; $P > 0.05$) nor $L_f$ ($r = -0.109$; $P > 0.05$) change as a result of ageing, whereas Morse et al. (2005a) revealed that both gastrocnemius lateralis muscle PPA (−13%) and $L_f$ (−16%) were significantly smaller with ageing. Briefly, the physiological implication of a shortened $L_f$ is a decrease in the number of sarcomeres in series, with a potential twofold effect: (i) an alteration to the working range of the muscle, where this unit may adapt by exhibiting a change in its force-length relationship, shifting to a shorter muscle length for peak force; and (ii) a decrease in the muscle-shortening velocity, and ultimately the muscle maximum power generation capacity. This cascade of effects would potentially cause problems for an obese or elderly population in activities such as locomotion and tasks involving the need to apply forces and relatively high velocities (such as getting up from a chair to answer the doorbell).

In the current study, the mean (across all BMI categories) GM muscle PPA during a maximum contraction decreased significantly with ageing (−8%), similar to the −16% ageing-related PPA decrease reported by Morse et al. (2005a), suggesting a loss of sarcomeres in parallel. A dissociation between fascicle length and pennation angle changes is not unique to the present study. For instance, a 12-month resistance-training program in the elderly highlighted increases in muscle PPA (12 vs. 19%) but no alterations in muscle $L_f$ (Morse et al. 2007).

### Muscle size

Prior to the present study, there appeared to be no information on the effect of body composition on PCSA. Our data, which employed an accurate, non-invasive measure of muscle size, revealed main effects of BMI ($P < 0.001$) and ageing ($P < 0.001$), as well as a BMI × age interaction ($P = 0.004$) for PCSA differences. Thus, we demonstrate that adiposity places a loading stimulus similar to that attained with resistance training in Y individuals (Erskine et al. 2010), more so than in O individuals (Morse et al. 2007) (Table 2). However, within the older cohort, the blunted response may be explained by the older muscle being unable to adapt to the load placed upon the musculature. These findings support the work by Lafortuna et al. (2013), who reported that absolute lower limb muscle volume increased along the continuum of increasing BMI from normal weight to obese individuals. However, Lafortuna et al. (2013) used a small sample ($n = 18$) as well as narrower age range (32–76-year-old females) in comparison with the present study.

In addition to the BMI × age interaction, the slopes of the regressions between BMI, body mass or adiposity and PCSA were steeper in Y vs. O (Table 3, Fig. 4B) individuals, thus highlighting the lower response to the loading effect from body mass/adiposity in the older cohort. The plasticity of the younger muscle appears to adapt structurally similarly to a resistance-trained muscle but the older musculature is unable to adapt to the loading. Reduced muscle mass is a known characteristic of sarcopenia in the elderly (Roubenoff, 1999) and is demonstrated in this study (−20% normal BMI O vs. normal BMI Y) even though the O females did not match the sarcopenic criterion ($9.6 \pm 1.5$ kg m$^{-2}$ in this group vs. ≤5.67 kg m$^{-2}$ standard; Baumgartner et al. 1998). However, the decreased GM PCSA was exacerbated in the obese O females (assuming a linear regression when compared with their underweight, normal weight and overweight counterparts). A plausible rationale for the greater loss in PCSA between Y and O obese individuals may be explained through higher levels of circulating pro-inflammatory cytokines seen in both obese and sarcopenic obese individuals (Hotamisligil et al. 1995; Schrager et al. 2007). Increases in inflammatory cytokines such as interlukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) have been shown to correlate negatively with muscle strength and lower muscle mass in the elderly (Visser et al. 2002). High levels of these specific cytokines expressed by adipose tissue seen in obesity (Schrager et al. 2007) are reported to increase catabolic activity of skeletal muscle (Roubenoff et al. 1997). In addition to increased catabolic activity, reduced anabolic signalling of growth hormones such as insulin-like growth factor-1 are reported in both elderly (Bucci et al. 2013) and severely obese male and females (Williams et al. 1984). Therefore, the potential synergistic action of increased catabolism and decreased anabolism may explain 'combined ageing and obesity'-induced losses in GM muscle tissue content, which are over and above expected 'normal ageing'-related decrements.

Future research would need to confirm the co-existence of high pro-inflammatory cytokine milieus, with decreased anabolic potential, in ageing-with-obesity. Based on such endocrine investigations into pro-inflammatory cytokines such as TNF-α and IL-6, it would then be possible to substantiate the interaction of the two factors (ageing and obesity),

in blunting the myogenic response associated with increased mechanical loading (in this case, through additional body fat), observed in this study.

## Conclusion

This study demonstrates for the first time that PCSA and FPA of the GM adapts to the loading stimulus of high BMI and/or adiposity in obese young and old females. Increases in GM PCSA and volume when correlated with either BMI and body or fat mass differed between the young and old obese females. The younger muscle mass was seen to adapt to the loading created by high levels of BMI and/or adiposity by increasing GM muscle volume and pennation angle to produce higher maximum torque. This adaptation, however, does not appear to occur in older obese persons. Nonetheless, the older cohort increased their FPA to the same extent as the young women, which may explain an increase in maximum torque in the obese old relative to other BMI/adiposity classifications of older women. These findings are suggestive of differential rate of skeletal muscle ageing, dependent on a person's body composition. Therefore, there is a case for implementing different exercise and/or nutrition interventions according to the somatotype and age of the individual concerned.

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### Conflict of interest

The authors confirm that they have no conflicts of interest to declare.

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ORIGINAL RESEARCH

# Obesity decreases both whole muscle and fascicle strength in young females but only exacerbates the aging-related whole muscle level asthenia

David J. Tomlinson, Robert M. Erskine, Keith Winwood, Christopher Ian Morse & Gladys L. Onambélé

Department of Exercise and Sport Science, Institute for Performance Research, Manchester Metropolitan University, Crewe Green Road, Crewe, CW1 5DU, U.K.

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**Correspondence**
David J. Tomlinson, Department of Exercise and Sport Science, Institute for Performance Research, Manchester Metropolitan University, Crewe Green Road, Crewe CW1 5DU, U.K.
Tel: (+44)0161 247 43665
Fax: (+44)0161 247 6386
E-mail: d.tomlinson@mmu.ac.uk

**Present Address**
Robert M. Erskine, Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, L3 3AF, U.K.

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**Abstract**

Obesity has previously been associated with greater muscle strength. Aging, on the other hand, reduces muscle specific force (the force per unit physiological cross-sectional area [PCSA] of muscle). However, neither the effect of obesity on skeletal muscle specific force nor the combined effects of aging and obesity on this parameter are known. This study aimed to describe the interplay between body mass index (BMI)/adiposity, aging, and skeletal muscle specific force. Ninety-four untrained healthy women categorized by age into young (Y; mean ± SD: 23.5 ± 9.0 years) versus old (O; 64.8 ± 7.2 years) were assessed for body composition, gastrocnemius medialis (GM) muscle volume (V), net maximum voluntary contraction (nMVC), and specific force (SF). The young obese, while demonstrating 71% and 29% ($P < 0.001$) higher V and nMVC compared to normal BMI individuals, were in fact 26% ($P = 0.007$) weaker than these, when V was used to scale nMVC (i.e., nMVC/V). The weakness associated with obesity was further exemplified in the 34% ($P < 0.001$) lower SF relative to normal BMI individuals. Similarly, >40% body fat was associated with 40% and 27% ($P < 0.001$) higher V and nMVC, but 11% and 25% ($P < 0.01$) lower nMVC/V and SF than <40% body fat. The aging-related rates of decline in V (−2 $cm^3$/year $P < 0.05$) and nMVC (−1.2 $cm^3$/year $P < 0.05$) were highest in obesity defined by BMI. This effect was also seen when aggregating by >40% adiposity. Interestingly, however, obesity appeared advantageous to the aging-related changes in nMVC/V ($P = 0.001$) and SF ($P < 0.001$). Unlike previous reports of greater strength in the obese compared with leaner age-matched counterparts, we in fact demonstrate that the young sedentary obese, are substantially weaker, where the volume of skeletal muscle is used to scale the maximal torque output, or forces are quantified at the fascicular level. The seemingly positive impact of obesity on rate of aging, however, is complex and warrants further investigations.

## Introduction

It is generally accepted that obese individuals, regardless of age, have lower maximal strength when the latter is expressed relative to body mass (Blimkie *et al.* 1990; Hulens *et al.* 2001; Rolland *et al.* 2004; Lafortuna *et al.* 2005; Maffiuletti *et al.* 2007, 2008; Abdelmoula *et al.* 2012). However, it is unclear whether this weakness exists at the fascicle level, or is simply a reflection of pseudo-hypertrophy whereby the relative high amount of muscle mass in an obese person is superseded by the higher elevation in adiposity. Previous studies have shown that the maximum strength capability relative to muscle size/mass of an obese individual is not significantly different to that of normal weight individuals (Blimkie *et al.* 1990; Lafortuna *et al.* 2005; Maffiuletti *et al.* 2007, 2008). This, how-

over, could be a potential type II error due to the use of total fat-free mass (Lafortuna et al. 2005; Maffiuletti et al. 2007, 2008) and anatomical cross-sectional area (ACSA; Blimkie et al. 1990) as indices of muscle size. This can be explained by the abovementioned measures not accounting for the architectural characteristic of the skeletal muscle responsible for the joint action, thus potentially misconstruing the true amount of muscle that contributes to torque production (Alexander and Vernon 1975). However, contradictions also exist in the literature with previous authors (Hulens et al. 2001) reporting that obese adult individuals have 6–7% lower torque relative to total fat-free mass and hypothesizing that this effect may be due to an assumed reduced agonist muscle activation. Indeed lower agonist muscle activation has been recorded in both obese adolescent (Blimkie et al. 1990) and obese adult populations (Tomlinson et al. 2014). Contrary to the above, other authors (Abdelmoula et al. 2012) reported obese adolescents to have 22% higher knee extension torque normalized to thigh lean muscle mass (data obtained from dual energy x-ray absorptiometry [DEXA]). In fact, the only study to have utilized a more accurate and valid method of measuring the muscle mass involved in isometric torque production was that of Hilton et al. (2008) who assessed the muscle volume of the triceps surae in obese individuals. These authors (Hilton et al. 2008) demonstrated that obese individuals produced lower torque relative to muscle volume. However, the participants recruited had diabetes mellitus and peripheral neuropathy, both of which have previously been shown to independently cause motor weakness (Andersen et al. 1996).

To our knowledge, to date, no obesity/strength interaction study has accounted for the pennate architecture of the antigravity musculature of the lower limbs in the estimations of muscle size. An accurate quantification of muscle size is its physiological cross-sectional area (PCSA; volume ÷ fascicle length), as this represents the number of sarcomeres in parallel, and exhibits a linear association with a muscle's maximum force capability (Fukunaga et al. 2001). Skeletal muscle specific force (force per unit PCSA) depicts an accurate representation of a muscle's maximum strength capacity, as it corrects for both the physiological and biomechanical determinants of maximal muscle strength (Maganaris et al. 2001; Reeves et al. 2004b; Erskine et al. 2009).

Aging has been shown to reduce skeletal muscle specific force (Morse et al. 2005). This reduction has been hypothesized as being due to a decline in the muscle activation capacity of elderly individuals, partly owing to lower habitual physical activity levels (Morse et al. 2004). In addition, preferential muscle fiber atrophy (Lexell and Taylor 1991) and a decrease in number of type II fibers (Lexell 1995) accompanied with an increase in intramuscular fatty infiltration (Rice et al. 1989), are likely to play a role in the aging-related lowering of muscle specific force. Yet, it appears that no investigation has systematically examined whether, after accounting for neural and architectural factors underlying muscle force production, the effects of aging would be exacerbated in the presence of obesity, thereby leading to a greater drop in maximal force generation capability of the old obese compared to their normal weight, age-matched counterparts. The increased prevalence of obesity (James 2008), which accompanies the rising level in life expectancy, renders the determination of therapeutic interventions to reverse the deleterious effects of the two "conditions" extremely timely.

Therefore, the aim of the present study was to investigate the degree of impact of obesity on skeletal muscle intrinsic force at both whole muscle and fascicular levels, in a young sedentary population. The second aim was to determine whether the effects of aging and adiposity are in fact additive on skeletal muscle specific force at both whole body and fascicle levels. We hypothesized that (1) skeletal muscle specific force in both obese Y and O would be lower when compared to lean, normal weight and overweight individuals, where muscle strength is quantified at both gross and fascicular levels; (2) the deleterious impact of high adiposity on skeletal muscle specific force (at both gross and fascicular levels), would be worse in the older individuals and their younger counterparts; and (3) the rate of aging (decrease in muscle contractile capacity) would be faster in the presence of obesity.

## Methods

### Ethical approval

Participants gave written informed consent prior to undertaking any assessment. All the procedures in this study had approval from the Manchester Metropolitan University Ethics committee and conformed to the standards set by the latest revision of the Declaration of Helsinki.

### Participants

The current study was on a single gender basis in order to minimize and the potential confounding effect of male versus female differential rate of aging (Lindle et al. 1997), and/or sensitivity to adiposity (Lafortuna et al. 2005, 2013). Thus, 94 untrained females volunteered to take part in this study (Table 1) and were categorized by age into either young (Y, 18–49 years) or old (O,

**Table 1.** Descriptive variables for body mass index (BMI) classifications in both the young (upper sections) and old (lower sections) age classifications.

| | Underweight | Normal | Overweight | Obese | BMI effect | Aging effect |
|---|---|---|---|---|---|---|
| *n* | | | | | | |
| Young | 13 | 52 | 8 | 18 | | |
| Old | 4 | 74 | 16 | 11 | | |
| BMI (kg/m$^2$) | | | | | | |
| Young | 18.8 ± 0.9 | 21.8 ± 1.1 | 27.8 ± 0.7 | 35.2 ± 4.5 | $P < 0.001$ | |
| Old | 19.1 ± 0.8 | 22.2 ± 1.0 | 27.4 ± 1.2 | 34.1 ± 3.7 | $P < 0.001$ | $P = n.s.$ |
| %Body fat | | | | | | |
| Young | 26.5 ± 3.8 | 30.1 ± 3.3 | 37.7 ± 5.8 | 45.5 ± 4.1 | $P < 0.001$ | |
| Old | 35.5 ± 2.1 | 35.8 ± 3.4 | 43.3 ± 2.8 | 46.1 ± 5.2 | $P < 0.001$ | $P = 0.002$ |
| Aging slope (mass (strength (Nm/s) year$^{-1}$) | 0.028 ($P = n.s.$) | −0.447 ($P = n.s.$) | −0.261 ($P = 0.01$) | 0.985 ($P = n.s.$) | | |
| Aging slope (specific force (Nm/s/$l$ year$^{-1}$) | 0.028 ($P = n.s.$) | −0.002 ($P = n.s.$) | 0.045 ($P = n.s.$) | 0.194 ($P = n.s.$) | | |

Data are presented as mean ± SD. Rate of aging per BMI classification is also shown (data are presented as regression slope and *P* value for the degree of association).

50–80 years). As 91/96 participants were white Caucasians, no subgrouping by ethnicity was carried out. Participants were then subcategorized into four body mass index classifications (BMI – body mass [kg]/stature$^2$ [m]) into underweight (BMI <20), normal (BMI 20–24.9), overweight (BMI 25–29.9), and obese (BMI >30). Participants were also categorized as ordinary adipose (<40%) versus high adipose (≥40%) by body fat percentage following recommendations from previous studies (Baumgartner et al. 2004; Rolland et al. 2009). The exclusion criteria were any health issues highlighted in the self-report questionnaire such as lower limb muscles/joints injuries/pathology, affecting mobility or ability to exert maximal torque. Physical activity status was screened by questionnaire and participants were excluded if they self-reported as habitually undertaking structured exercise for more than 3 h per week.

## Protocol order

Participants attended the laboratory for testing on two occasions. During the first visit, anthropometric measurements (DEXA, Stature, Mass) were collected, and familiarization with the MVC protocols took place. During the second visit participants gastrocnemius medialis muscle volume and architecture data were collected alongside the main MVC protocol.

## Body composition measure

Body composition (body fat percentage, lean muscle, and bone) was ascertained using a DEXA scanner (Hologic Discovery; Vertec Scientific Ltd, Reading, U.K.) following a period of overnight fasting for 12 h. Participants lay in the supine position, avoiding any contact between the trunk and the appendicular mass during a 7-min scanning procedure (whole body procedure, effective dose 8 μSv). Scan results were both graphical (Fig. 1) and numerical, giving a number of descriptions of which % body fat was key for the aims in this study.

## Muscle strength measurement

Maximum voluntary contraction (MVC) torque during both ankle plantar flexion (PF) and dorsi flexion (DF) was measured in the dominant limb using an isokinetic dynamometer (Cybex Norm; Cybex International, New York, NY). Participants were seated (hip at 85° angle, dominant leg extended with the foot secured to the footplate of the dynamometer), and strapped using inextensible straps (at the hip, distal thigh, and chest) to reduce extraneous movements. Prior to MVCs, participants were refamiliarized with the protocol undertaken during the test. Following this familiarization protocol, participants conducted a series of five submaximal isometric contractions with their ankle positioned at 0° (anatomically neutral), starting at self-perceived 50% maximal exertion, increasing in intensity to ensure the participant was warmed up prior to maximal exertion.

During the main MVC protocol, participants were asked to conduct two (up to a maximum of four, see below) rapid isometric PF and DF MVCs with their ankle positioned at 0°, each lasting 3–4 sec. The highest (of two) recorded PF and DF MVC by the participant was utilized as their true MVC. However, MVCs were repeated if there was >10% difference between MVCs to

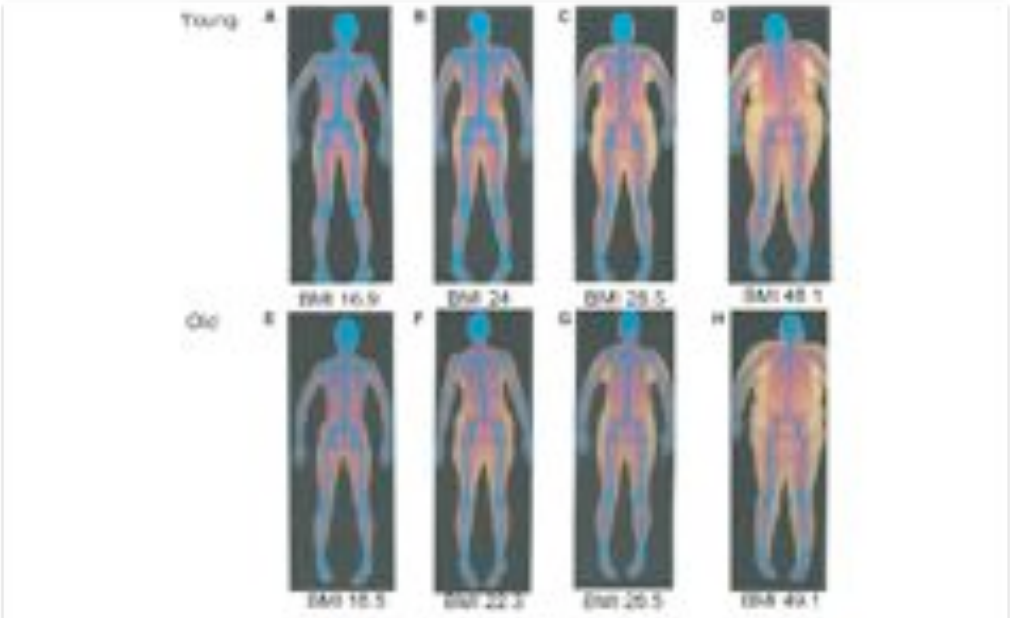


**Figure 1.** Representative dual energy x-ray absorptiometry (DEXA) scans of a (A) young underweight female, (B) young normal weight female, (C) young overweight female, (D) young obese female, (E) old underweight female, (F) old normal weight female, (G) old overweight female and (H) old obese female. Color key: blue for bone, red for lean tissue, yellow for adipose tissue.

ensure true MVC was obtained. The PF MVC was then corrected for agonist muscle activation using the interpolated twitch technique (Morse et al. 2004; Pearson and Onambele 2006, Fig. 2) and antagonist coocntraction of the tibialis anterior (TA) using surface electromyography (EMG). Antagonist muscle coocntraction was calculated through utilizing the EMG signal (computed as root mean square) of the TA recorded 500 msec on either side of instantaneous peak torque during a maximal PF and divided by the EMG recorded during DF. The raw EMG signal measured during contractions was recorded at 2000 Hz, with band pass filter set at 10–500 Hz, and notch at 50 Hz. This calculation method assumes that the DF EMG/Torque relationship is linear (Maganaris et al.

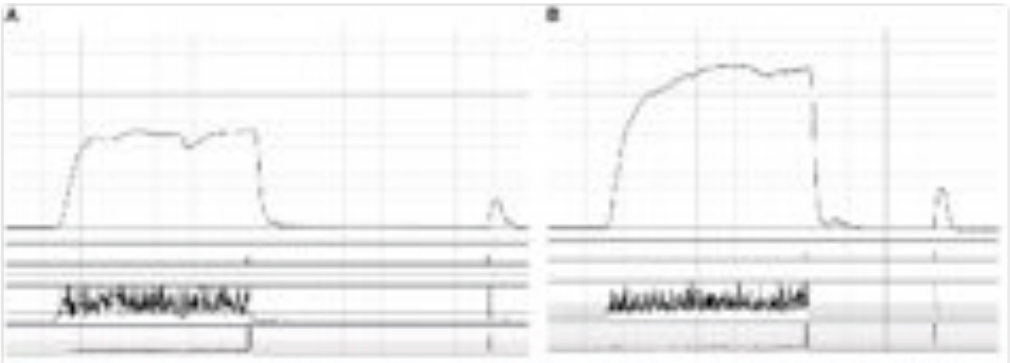


**Figure 2.** Representative torque outputs taken during the interpolated twitch technique of a (A) young ordinary-adipose female and (B) young high-adipose female.

1998). MVCs corrected for both agonist muscle activation and antagonist co-contraction (i.e., neural factors) in the manuscript are classified as net MVC (nMVC).

## Muscle volume and intrinsic strength

Participants lay in the prone position with their ankle positioned at 0°. B-mode ultrasonography was then used to ascertain the origin and insertion of the GM, where discrete muscle sites (25%, 50%, and 75% of length) were marked from the medial to lateral border of the GM. Thin strips (2 mm) of micropore tape (3M, Bracknell, Berkshire, U.K.) were placed axially 3–4 cm apart transversally along the nominated muscle lengths. The micropore tape was utilized as echo-absorptive marker in the formation of ACSAs from the corresponding muscle lengths when using the photo-editing software (Adobe Photoshop Elements; Version 10, Adobe Systems Incorporated, San Jose, CA). During recording of the ACSAs, the ultrasound probe (7.5 MHz linear array probe, 38-mm wide) was held perpendicular to the GM on its medial border and moved along a designated marked pathway to its lateral border. The probe was moved steadily across the leg with constant light pressure to avoid compression of the muscle during scanning. This procedure was repeated twice at each discrete muscle site for reliability purposes. The construction of the ACSAs were undertaken using Adobe Photoshop (Version 10), where still transverse images at each individual muscle length were reconstructed using the micropore tape as anatomical markers in combination with anatomical landmarks along the GM muscle length. Following construction of the three individual ACSAs, the areas of the complete transverse ACSAs were undertaken using the analysis software ImageJ (1.43u; National Institutes of Health, Bethesda, MD). Reliability in the measure of the three ACSAs was assessed in 10 participants (Y = 5; O = 5; BMI range = 17.6–36.7) on two separate days (separated by at least 48 h) by the same investigator. The intraclass coefficients were as follows: GM ACSA 25% length – 0.998, GM ACSA 50% length – 0.999, GM ACSA 75% length – 0.998. These measures are not only reliable but also externally valid as they demonstrate strong agreement with MRI-obtained values (Reeves et al. 2004a). Muscle volume was then calculated using the truncated cone method:

$$\text{Cone volume} = [1/3 \times h] \times \pi \times (R_1^2 + R_1) \times (R_2^2 + R_2)$$

Where $R_1$ = radius of the base, $R_2$ = radius of the top, h = distance between segments; $R = \sqrt{(\text{ACSA}/\pi)}$, where $\pi = 3.142$.

Skeletal muscle intrinsic strength was quantified as nMVC relative to muscle volume.

## Calculation of GM-specific force

This involved several steps including the assessment of tendon force (itself requiring measurement of tendon moment arm) and muscle physiological cross-sectional area (itself requiring the assessment of muscle architecture).

### Tendon moment arm

Tendon excursion using B-mode ultrasonography was used to calculate the Achilles tendon moment arm length (Ito et al. 2000; Maganaris et al. 2000). Participants were seated in the isokinetic chair (Cybex Norm; Cybex International) following the experimental set up undertaken during MVC's. Prior to commencement of the protocol, the insertion of the GM muscle to the Achilles tendon was anatomically marked on the limb using micropore tape as an echo-absorptive reflective marker during recording. The ultrasound probe was then positioned across the muscle–tendon junction (MTJ) of the GM as denoted by the micropore tape. During the recording the ankle of the participant was passively rotated between 10° and −5° PF at a constant velocity of 1°/sec. The passive movement was recorded for at least three PF and DF rotations and was synchronized with torque outputs using a square wave signal generator to distinguish joint position on the capturing software.

The displacement of the MTJ of the GM between 10° PF and −5° PF was calculated using the micropore tape as a distance marker using analysis software (ImageJ 1.43u; National Institutes of Health). The Achilles moment arm length at 0° was then calculated using the displacement of the MTJ divided by the displacement in the ankle angle during a complete rotation.

$$\text{MA}_{0^\circ} = \text{displacement MTJ} \div \text{change in ankle angle}$$

### Tendon force

Achilles tendon force (F) at 0° was calculated using the PF MVC corrected for both agonist muscle activation and antagonist cocontraction, then dividing this value by the Achilles tendon moment arm length. The contribution of the GM muscle to PF MVC was calculated, assuming this muscle to contribute 25% of the total ankle plantar flexion MVC (Fukunaga et al. 1992).

$$F_{\text{GM}} = F \times 0.25, \text{where } F = \text{nMVC} \div \text{MA}_{0^\circ}$$

### Muscle architecture

Muscle architecture of the gastrocnemius medialis (GM) was measured using B-mode ultrasonography at both rest and during a graded isometric MVC over 6 sec. Partici-

punts were seated in an isokinetic dynamometer (Cybex Norm; Cybex International) as detailed above. The probe of a B-mode ultrasound scanner (AU5 Harmonic; Esaote Biomedica, Genoa, Italy) was positioned on the surface of the skin, at 50% of the GM muscle length, along the mid-sagittal line. Participants were then asked to perform a ramped MVC over 6 sec, where the change in both fascicle pennation angle (PFA) and fascicle length (Lf) were recorded. Images of both resting and maximal architecture were synchronized with torque outputs using a square wave signal generator, extrapolated from the capturing software (Adobe Premier pro Version 6; Adobe Systems Software, San Jose, CA) and later analyzed using ImageJ (1.47i; National Institutes of Health). Analysis of three fascicles defined from the deep to the superficial aponeurosis of the GM was then recorded and the mean value of both the PFA and Lf of the three fascicles were then recorded. If needed (in cases where the fascicles extended beyond the width of the probe), linear extrapolation of fascicles was carried out. The reliability of the measure of both PFA and Lf at rest and MVC was obtained from 10 participants (Y = 5; O = 5; BMI range = 17.6–36.7). The intraclass correlation coefficients for both the architectural measurements were high (muscle fascicle pennation angle rest – 0.997, muscle fascicle pennation angle max – 0.997, muscle fascicle length rest – 0.996, muscle fascicle length max – 0.993).

Following the calculation of GM muscle volume and architecture, PCSA was then calculated (PCSA = Muscle volume ÷ Lf).

### Fascicle force

Following the computation of the GM muscle force ($F_{GM}$), fascicle force was calculated as

$$GM\,F_{fas} = F_{GM} \div \cos \mathrm{PFA}$$

Gastrocnemius medialis muscle specific force was calculated by dividing the GM fascicle force by GM PCSA (Alexander and Vernon 1975; Fukunaga et al. 1996; Reeves et al. 2004b).

$$GM\,SF = GM\,F_{fas} \div \mathrm{PCSA}$$

## Statistical analyses

Statistical analyses were carried out using SPSS (Version 19, SPSS Inc., Chicago IL). Stem-and-Leaf plots were used to identify any outliers, and these were removed prior to further analyses. To determine parametricity, Shapiro–Wilk (normal distribution) and Levene's tests (homogeneity of variance) were used. If parametric assumptions were met, a one-way analysis of variance (ANOVA; BMI classifications) with post hoc Bonferroni correction for pairwise comparisons, or independent sample *t*-tests (% body fat classifications) were used on muscle volume, nMVC, GM intrinsic strength, and GM specific force (GM SF). Where parametric assumptions were breached, Kruskal–Wallis *H* (BMI classifications) or Mann–Whitney *U* (%body fat classifications) were used. Linear regressions and Pearson's moment correlations, described the relationships and the degree of association, between age and parameters of interest (including muscle volume, nMVC). Comparisons of the regression coefficients and slopes were conducted using *z*-transformations and the Student's *t*-statistic. Data are reported as mean ± SD and statistical significance was accepted when $P \leq 0.05$.

# Results

## Body composition

Tables 1 and 2 display descriptive study population characteristics of BMI and body fat% for Y and O females categorized by both BMI and %body fat.

## Skeletal muscle characteristics

### BMI and muscle contractile characteristics

Figure 3 demonstrates the effect BMI classification has upon muscle volume, nMVC torque, intrinsic strength, and specific force in young participants.

Muscle volume revealed a main effect of BMI classification ($P < 0.001$). Pairwise comparisons revealed the obese females to have 75% ($P < 0.001$), 71% ($P < 0.001$), and 36% ($P < 0.010$) greater muscle volume than their underweight, normal weight and overweight counterparts, respectively.

Net MVC torque revealed a main effect of BMI classification ($P < 0.001$). Pairwise comparisons revealed the obese females to have 39% ($P < 0.001$) and 29% ($P = 0.005$) greater nMVC than their underweight and normal weight counterparts, respectively.

The opposite ranking order was found for indices of strength normalized for muscle content. Indeed intrinsic strength revealed a main effect of BMI classification ($P = 0.005$). Pairwise comparisons revealed the obese females to have 26% ($P = 0.007$) lower intrinsic strength than their normal weight counterparts. However, there was no difference in intrinsic strength between the young obese and either underweight, or overweight individuals (Fig. 3C).

Skeletal muscle specific force (GM SF) revealed a main effect of BMI classification ($P = 0.002$). Pairwise comparisons revealed the obese females to have 40%

**Table 2.** Descriptive variables for obesity classification by body fat percentage in both the young (upper section) and old (lower section) age classifications.

| | Ordinary adipose (n = 16) | High adipose (n = 17) | Obesity effect | Aging effect |
|---|---|---|---|---|
| n | | | | |
| Young | 12 | 17 | | |
| Old | 19 | 20 | | |
| BMI | | | | |
| Young | 21.9 ± 2.9 | 36.5 ± 5.0 | $P < 0.001$ | |
| Old | 24.4 ± 3.5 | 39.6 ± 5.8 | $P < 0.001$ | $P = 0.463$ |
| %Body fat | | | | |
| Young | 29.9 ± 5.1 | 40.9 ± 3.5 | $P < 0.001$ | |
| Old | 33.6 ± 4.7 | 44.6 ± 4.7 | $P < 0.001$ | $P = 0.008$ |
| Aging slope intrinsic strength ($N.m.yr^{-1}$) | −0.278 ($P = 0.01$) | −0.018 ($P = n.s.$) | | |
| Aging slope specific force ($N.m^2.year^{-1}$) | −0.018 ($P = n.s.$) | 0.173 ($P = n.s.$) | | |

Data are presented as mean ± SD. Rate of aging per adiposity classification is also shown (data are presented as regression slope and *P* value for the degree of association).

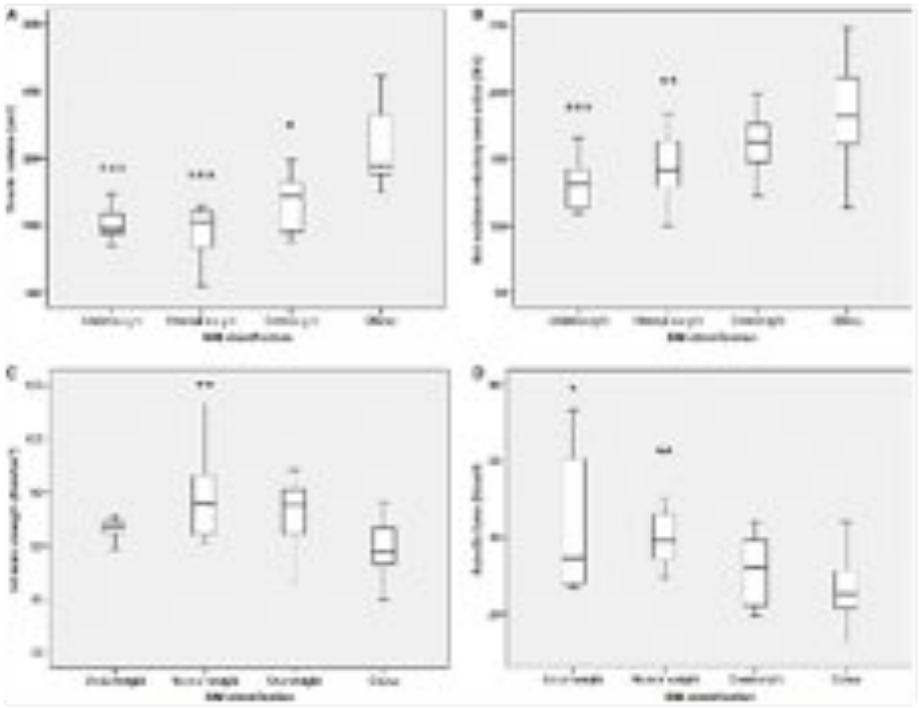


**Figure 3.** Displays the impact of body mass index (BMI) classification on (A) muscle volume (1–3 overweight and 2 obese outliers), (B) net maximum voluntary contraction (3 outliers), (C) intrinsic strength (1–3 underweight and 2 normal weight outliers) and (D) specific force (1–3 normal weight outliers) in young females ($NP = 0.05$, $NP = 0.01$, $***P = 0.001$).

($P = 0.006$), 34% ($P = 0.010$) lower specific force than their underweight and normal weight counterparts, respectively. There was no difference in GM SF between overweight and obese young individuals.

### Adiposity and muscle contractile characteristics

Figure 4 demonstrates the effect that obesity classification by adiposity (i.e., %body fat) has upon muscle volume, nMVC, intrinsic strength, and specific force in young participants.

Highly adipose females were found to have 60% ($P < 0.001$) greater muscle volume, and 27% ($P < 0.001$) greater nMVC than "ordinary" adipose females.

Interestingly, however, as seen in BMI classifications, this seeming advantage of high adiposity was reversed when it was shown that the high-adipose females had in fact 11% ($P = 0.025$) lower intrinsic strength, and 25% ($P = 0.005$) lower specific force than their age-matched ordinary adipose-matched females than the ordinary classified females.

### Degree of association between age and muscle size and/or strength by obesity status

There was a stepwise increment in the steepness of the aging versus muscle content loss relationship, with increasing BMI. Thus, aging-related muscle loss from the second to the seventh decade were −2, −0.5, 0.2, and 0.3 $cm^3$/year in the obese, overweight, normal weight, and underweight BMI categories, respectively. These differences in slopes were significant between obese and normal weight (Student's $t$ statistic 3.88; $P < 0.01$), obese and underweight (Student's $t$ statistic 3.64; $P < 0.05$), and obese and overweight (Student's $t$ statistic 2.59; $P < 0.05$) females (Fig. 5A).

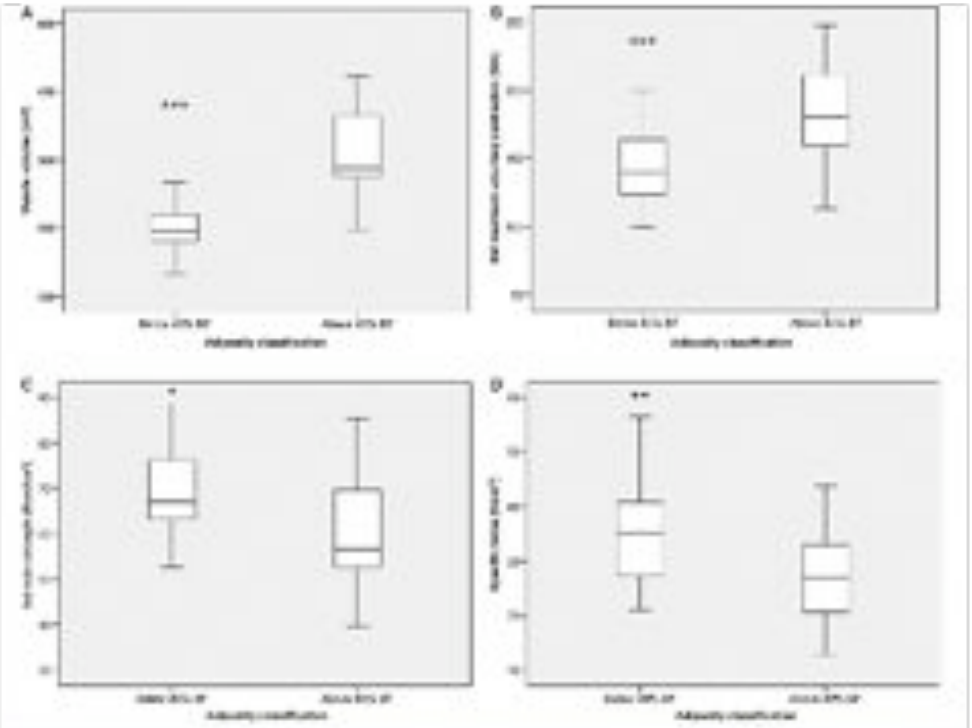

**Figure 4.** Displays the impact of adiposity on (A) muscle volume (− 1 overweight and 1 obese outliers), (B) net maximum voluntary contraction (− 1 overweight outlier), (C) intrinsic strength (− 3 underweight and 2 normal weight outliers) and (D) specific force (−1 underweight, 1 normal weight, and 1 overweight outliers) in young females (* = 0.05, ** = 0.01, *** = 0.001).

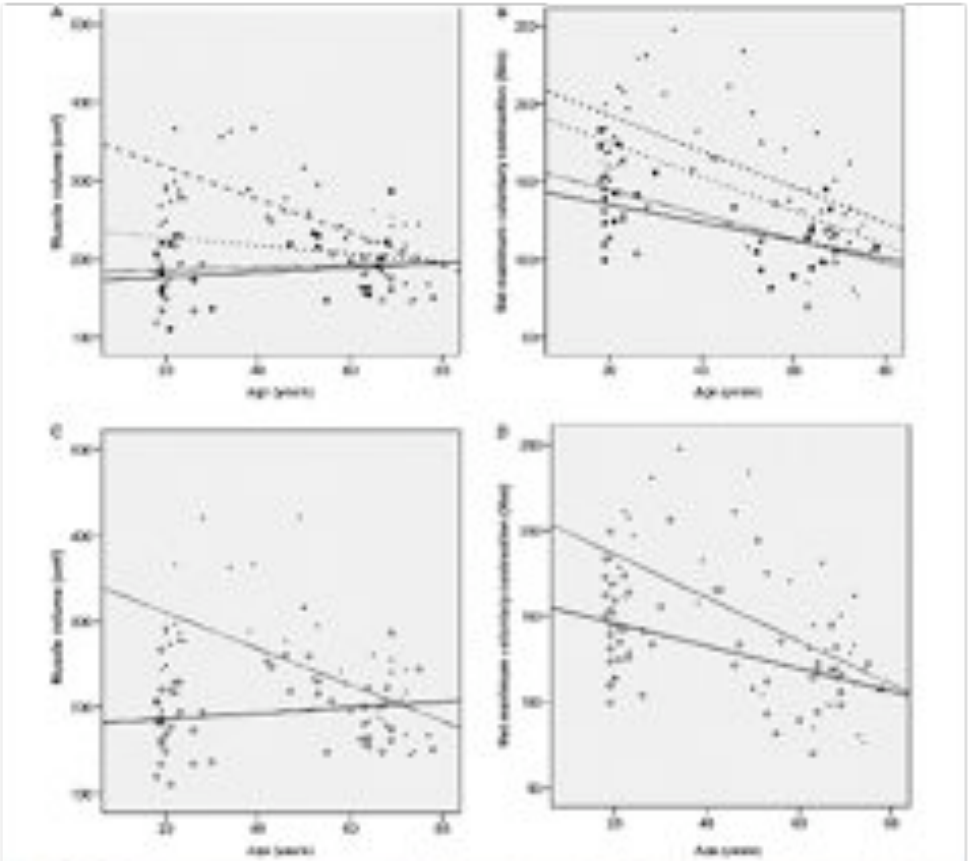

**Figure 5.** Displays linear regressions (and slope comparisons) on the impact of age on both BMI and adiposity classification on muscle volume (A, C) and net maximum voluntary contraction (B, D) in sedentary females. (A) Underweight (□ ——□) versus Obese (▲ – – –) ($P < 0.05$). Normal weight (● · · · · ·) versus Obese, $P = 0.05$). Overweight (○ - - - -) versus Obese $P = 0.05$). (B) Underweight (□) versus Obese (▲) ($P = 0.05$). Normal weight (●) versus Obese, $P = 0.05$). Overweight (○) versus Obese $P = 0.05$). (C) Ordinary adipose (□ ——□) high adipose (· · · · · ·) $P = 0.05$). (D) Ordinary adipose versus high adipose, $P = 0.05$). Briefly, in terms of muscle volume, the obese individuals were shown to significantly decrease muscle mass per year at a fast rate (−2.1 $cm^3$/year, $P < 0.001$) than overweight (−0.5 $cm^3$/year $P = n.s$), normal weight (0.2 $cm^3$/year, $P = n.s$), underweight females (0.3 $cm^3$/year, $P = n.s$) or ordinary adipose females (−2.1 $cm^3$/year $P < 0.001$ vs. −0.3 $cm^3$/year $P = n.s$). In terms of nMVC, there was a nonsignificant trend for the obese individuals to lose maximum strength capability at a slightly greater rate (−1.2 Nm/year, $P = 0.019$) than their overweight (−1.1 Nm/year, $P = 0.002$), normal weight (0.8 Nm/year, $P = 0.008$), underweight females (0.6 Nm/year, $P = n.s$) or ordinary adipose females counterparts (−1.8 Nm/year, $P < 0.001$ vs. 0.7 Nm/year, $P < 0.001$).

Similarly, there was a stepwise increment in the steepness of the aging versus muscle content loss, with high adiposity. Thus, aging-related changes from the second to the seventh decade were −2.1 versus 0.3 $cm^3$/year in high versus low %body fat categories. These differences in slopes were statistically significant (Student's $t$ statistic 4.42; $P < 0.05$; Fig. 5C). Based on these slopes, it was evident that for muscle volume aging was associated with faster than normal deleterious changes, regardless of whether classification was by BMI or by fat.

There was a stepwise increment in the steepness of the aging versus nMVC loss relationship, with increasing

BMI. Thus, aging-related changes from the second to the seventh decade were −1.2, −1.1, 0.8, and 0.6 Nm/year in the obese, overweight, normal weight, and underweight BMI categories, respectively. These differences in slopes, were significantly different between obese and normal weight (Student's $t$ statistic 3.88, $P < 0.05$), obese and underweight (Student's $t$ statistic 3.64, $P < 0.05$), and obese and overweight (Student's $t$ statistic 2.59, $P < 0.05$) females (Fig. 5A).

Similarly, there was a stepwise increment on the steepness of the aging versus nMVC loss relationship, with high adiposity. Thus, aging-related changes from the second to the seventh decade were −1.3 versus 0.7 Nm/year in high versus low %body fat categories. However, similar to the BMI classification, this difference in the slope was statistically significant (Student's $t$ statistic 4.82, $P < 0.05$; Fig. 5C).

As expected, there was a significant decrease in intrinsic strength with aging (−0.17 N/cm$^2$/year, $P = 0.027$). Grouping by BMI, only highlighted a significant change with aging in the overweight (see Table 1). Grouping by adiposity highlighted a significant decrease in the low-adipose group but no change in the high-adipose group (see Table 2).

Unexpectedly, there was no change in specific force with aging ($P > 0.05$). Also, grouping by BMI or adiposity highlighted no subgroup effect (see Tables 1 and 2).

## Discussion

This study is the first to systematically quantify the impact of varying levels of BMI and adiposity on skeletal muscle intrinsic (i.e., whole muscle level) and specific (i.e., fascicle level) force. When body composition status was classified by BMI, the obese cohort exhibited greater muscle volume and nMVC. Interestingly, however, the obese (compared to normal weight and underweight individuals) in fact had lower nMVC normalized to muscle volume, as well as lower muscle specific force. This significant trend was repeated when obesity was classified using adiposity, as the high-adipose female cohort (≥40% body fat) demonstrated both higher muscle volume and nMVC, yet lower nMVC normalized to muscle volume and specific force. These findings supported our first hypothesis.

The rate of aging in terms of both muscle volume and nMVC was found to be worst in the obese cohort whether classified by BMI or adiposity, again supporting one of our hypotheses. However, contrary to our final hypothesis, a decrease in intrinsic strength was only apparent in the pooled population (regardless of obesity status). Where the data were grouped by BMI, only the overweight showed an aging-associated decrease in intrinsic strength. No aging changes were significant with the population grouped by adiposity. Specific force was not affected by aging in the pooled population nor where the population was grouped either by BMI or adiposity.

### The effect of BMI and adiposity on muscle contractile characteristics

The classification of obesity in the majority of previous studies investigating the effect of high levels of adiposity on muscle structure and function has been through BMI (Hulens et al. 2001, 2002; Rolland et al. 2004; Maffiuletti et al. 2007, 2008; Delmonico et al. 2009). However, BMI does not distinguish between fat, lean, and bone mass in the calculation of obesity and instead utilizes an individual's body mass relative to their height. We argued that this had the potential to conceal the true effect that differing levels of adiposity may have on skeletal muscle properties (Rothman 2008). Therefore, the results of the current study were categorized by both BMI and body fat percentage to determine whether one or the other obesity classification strategy would be the most powerful in distinguishing between the populations (Baumgartner et al. 2004; Rolland et al. 2009).

The two obesity classifications (i.e., by BMI or adiposity) provided comparable conclusions with regard to the reported magnitude of group differences in muscle contractile characteristics between obese and nonobese classes. This single conclusion supports the hypothesis that obesity does indeed loads the antigravity musculature in a manner similar to simulated hypergravity (Bosco et al. 1986; Klentrou et al. 2007) and/or resistance training. Indeed our data shows an increase in both nMVC and muscle volume in the adult, young obese individuals. For instance, if primarily focusing on obesity effect on nMVC (net total muscle strength), there was a significant greater maximum torque whether participants were classified obese by BMI (+29%) or adiposity (+27%). Our results concur with previous research findings of +12% (Hulens et al. 2001) and +16.5% (Maffiuletti et al. 2007) significantly greater absolute knee extensor torque in obese compared with normal weight individuals. However, with the aforementioned two studies, the recorded maximum torque was not corrected for agonist muscle activation or antagonist coactivation, leading to an underestimation of the true torque of the obese individuals. Indeed, young obese adults have been demonstrated to have significantly lower (92% vs. 85%) agonist muscle activation capacity compared to age-matched normal weight counterparts (Tomlinson et al. 2014).

Similarly, obesity was associated with increased GM muscle volume when compared to normal weight (+71%) and ordinary adipose (+60%) classified females. Arguably,

this further supports the idea that obesity in the young adult, potentially leads skeletal muscle similar to progressive resistance training (Erskine et al. 2010). This theorem has been supported by Lafortuna et al. (2013) who demonstrated a positive association between adiposity and lower limb skeletal muscle volume using computed tomography in adult females. However, no other study, prior to our present work, had accurately quantified the musculature involved in the specific joint movement of interest, through accounting for the volume and architecture of said musculature.

While the above two parameters (i.e., nMVC and muscle volume) suggest a positive impact of obesity, when normalizing maximum torque to the individual's muscle volume (intrinsic strength), a maladaptation of the skeletal musculature to obesity becomes evident. This is illustrated both when individuals were classified obese (through BMI (−26% lower intrinsic strength) and adiposity (−11% lower intrinsic strength). Our results suggest that at the whole muscle level, young adult obese individuals irrespective of obesity classification are at a disadvantage relative to normal weight individuals. Interestingly, when comparing these results to previous work, there appears to be conflicting reports within the literature. On one hand, some researchers (Hulens et al. 2001) reported MVC knee joint torque normalized to total fat-free mass to be 6–7% significantly lower in obese females. These authors hypothesized lower agonist muscle activation as being the physiological basis of this obesity effect. However, other authors (Maffiuletti et al. 2007) reported the differences between obese and non-obese muscles to disappear when torque was normalized to total fat-free mass. Arguably, muscle volume is a more accurate measure of muscle size than total fat-free mass (Akagi et al. 2009), hence direct comparison of the results from the literature is meaningless, potentially explaining the conflicting reports.

While normalizing nMVC to muscle volume gives an accurate depiction of the whole muscle level features (i.e., the intrinsic strength), skeletal muscle specific force describes characteristics at fascicle level. The calculation of skeletal muscle specific force accounts for the physiological and biomechanical determinants of a muscles force generating capacity (Maganaris et al. 2001). The fascicle level results from our current study are in-line with the above described whole muscle level findings. The obese young females classified by both BMI and adiposity demonstrated significantly lower (−33% and −23%, respectively for each classification method) skeletal muscle specific force compared to normal weight individuals. Since this is the first study to control for the neural, morphological, and biomechanical factors in force generation, there are no data at a fascicle level to compare our data against. Nonetheless, the underlying mechanisms may potentially be linked to higher levels of inflammation often measured in obese individuals (Hotamisligil et al. 1995) and an increase in fatty infiltration within the muscle (Hilton et al. 2008), both likely to lower the intrinsic and specific strength potential of the obese skeletal musculature.

## Impact of BMI and/or adiposity on the magnitude of aging-related sarcopenia and asthenia

When rate of aging was determined by populations for changes in muscle volume, nMVC, intrinsic strength, and specific force, the fastest changes were seen with muscle volume and nMVC (as seen in Fig. 5). However, it was apparent that the greatest effect of combined adiposity and aging was seen in the rate of loss of muscle volume (Fig. 5A and C). One of the mechanisms that can underpin the faster loss of muscle mass may be the cumulative effect of higher inflammation observed in both obese and elderly individuals (Cesari et al. 2004; Park et al. 2005; Schrager et al. 2007; Degens 2010), coupled with a lower anabolic profile both in old age (Bucci et al. 2013) and obesity (Frost et al. 2003; Galli et al. 2012). This enhanced susceptibility to sarcopenia in the obese, emphasizes the deleterious impact of this condition, hence highlighting the importance of maintaining a healthy body composition.

The effect of aging on nMVC was significant in old subject groups. However, there were no significant differences between the slopes of the regressions between obesity categories (see Fig. 5B and D). The importance of this finding stems from a further exacerbation of an existing relatively low strength to body mass ratio in obese individuals (Blimkie et al. 1990; Zoico et al. 2004; Lafortuna et al. 2005; Maffiuletti et al. 2007, 2008), thereby rendering daily functional capacity even more compromised, with the ability to carry out tasks such as rising from a chair or squatting deep to reach items on the floor for instance, quicker to lose. Arguably, a more detailed description of decade-by-decade changes on sensitivity to adiposity would be warranted in future studies, as would the additional consideration of ethnicity.

Interestingly, in the current study population, the effect of aging on either intrinsic strength or specific force (when categorized by BMI or adiposity) was as expected, as no significant changes were observed. Notably, however, aging was associated with a significant decrement in intrinsic strength in the pooled population. Our data partially support previous reports that have shown specific force to decrease with age (Morse et al. 2005). Interestingly, resistance training has been shown to

increase specific force in the elderly (Morse et al. 2007). Therefore, assuming the additional fat mass seen in obesity chronically loads the skeletal muscle to a degree similar to resistance training, together with the extended proportion of their lifespan as obese individuals (Abdullah et al. 2011), may explain the lack of deleterious aging-related changes in intrinsic strength and muscle specific force, even in this population. Potentially such an effect would be mediated via a shift in muscle fiber-type composition toward type II fibers. Indeed such fiber-type composition bias has previously been reported in obese individuals (Krotkiewski et al. 1983).

The lack of aging-related decrease in three out of four BMI or adiposity subgroups in the current population, may also be indicative of a healthy older population, as demonstrated through their health questionnaire data.

## Conclusion

Our study demonstrates for the first time that at both whole muscle and fascicular levels, high BMI or adiposity categories of obesity are associated significantly with lower skeletal muscle contractile capacity in young adults. Interestingly, the aging effect on obese individuals classified by both BMI and adiposity was foremost observed through the loss of muscle tissue content as well as total muscle strength. The dissociation in the aging-related rate of changes in the BMI and/or adiposity categories meant that in the presence of obesity, aging did not lower skeletal muscle intrinsic strength and/or muscle specific force. While this latter finding warrants further investigations, our results suggest that obesity even where individuals are recreationally active (as in the present study sample), should be targeted using therapies aimed at minimizing sarcopenia and asthenia in later life.

## Acknowledgments

The authors are ever indebted to every one of the participants in this study for their time and adherence to the protocol conditions. Additionally, the authors would like to acknowledge the work of Fay Manning and Adam Bentley for their assistance in data collection. D. Tomlinson is the postgraduate student who carried out the day-to-day experiments, data analyses, and produced the first manuscript draft. R. Erskine, K. Winwood, and C. Morse are members of the supervision team for D. Tomlinson and were instrumental in the study design, protocol refinements, and data interpretation. G. Onambele is the director of studies, who trained D. Tomlinson, finalized the study design and protocols, and oversaw data analyses as well as all manuscript drafts.

## Conflict of Interest

None declare.

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ERRATUM

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# Obesity decreases both whole muscle and fascicle strength in young females but only exacerbates the ageing-related whole muscle level asthenia

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In the calculation of gastrocnemius medialis (GM) fascicle force, the tendon force was divided by the pennation angle rather than the cosine of the pennation angle, thus leading to an overestimation of GM fascicle force and GM specific force by approximately 30% on average, with heteroscedastic distribution in the individual overestimates.

Thus, we need to refine our concluding remarks to the effect that:

A) With BMI classification, obesity did not impact on muscle specific force ($P = 0.120$), (previously specific force was reported as significantly lower in the obese group ($P < 0.001$) compared to that in normal weight counterparts). Please see amended Figures 3D and 4D below.
B) With adiposity classification, the results remain as previously reported except that the specific force of the high adiposity group was not significantly lower ($P = 0.129$) than that seen in the low adiposity group (previously this was reported as significantly lower ($P < 0.011$).
C) There was no impact of obesity (either by BMI or adiposity) on the slope of age related changes in terms of specific force.

The lack of impact of obesity on rate of specific force change with increased ageing is complex and warrants further investigations.

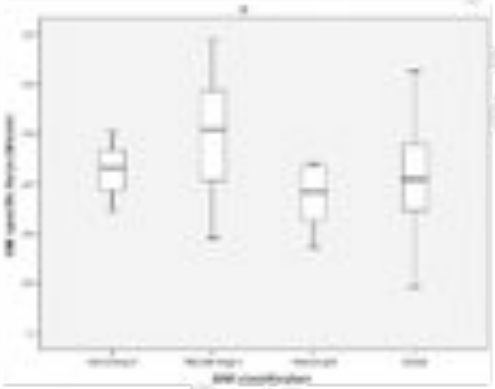

Figure 3D

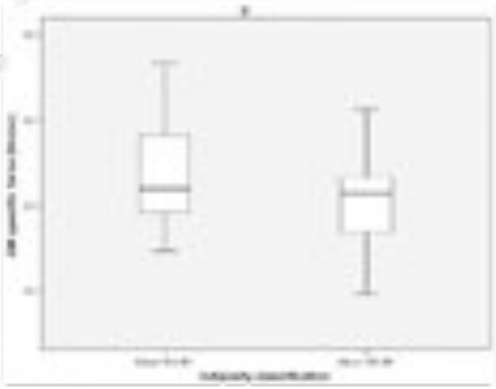

Figure 4D

### The Effect of Adiposity on Skeletal Muscle Size and Strength in Untrained Women

Tomlinson D.J[1]., Morse C.I.[1], Winwood K.[1], Erskine R.M[1,2]. and Onambele G.L[1].

*[1]Institute for Performance Research, Department of Exercise and Sport Science, Manchester Metropolitan University, Crewe Green Road, Crewe, CW1 5DU, UK. [2]Current address: Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, L3 3AF, UK.*

Background: It is not known how elevated adiposity in obese individuals or abnormally low levels of adiposity in underweight people affects the size and strength of skeletal muscle. Resistance training is known to induce gains in both muscle size and strength (Erskine *et al*., 2010), while prolonged muscle disuse has been shown to cause a reduction in these variables (de Boer *et al*., 2007). Moreover, simulated hypergravity for a 3 week period has been shown to increase muscle strength (Bosco *et al*., 1984), while low-load, high volume resistance exercise has been shown to enhance muscle protein synthesis more than conventional high-intensity resistance exercise (Burd *et al*., 2010). Therefore, we hypothesised that chronic over-loading of skeletal muscle in obese individuals would lead to greater lower-limb lean mass and higher absolute strength values compared to under-weight and normal-weight individuals.

Method: Fifty untrained healthy women aged 19-49 yrs (mean ± SD: 25.1 ± 8.7 yrs) were recruited to take part in this study. Body fat and lean mass were assessed using dual-emission X-ray absorptiometry. Plantar flexion maximum voluntary isometric contraction (MVC) torque was assessed in the dominant limb using an isokinetic dynamometer at different ankle joint angles (-5 to +10 deg plantar flexion; 0 deg = neutral ankle position). MVC joint torque was corrected for voluntary muscle activation level (assessed using the interpolated twitch technique) and antagonist muscle co-activation (assessed via surface EMG).

Results: Body mass index was 26.0 ± 7.0 kg/m$^2$ (range: 17.0 – 51.9 kg/m$^2$. Body fat percentage was 35.4 ± 8.8% (range: 19.6 – 51.9%). There was a positive relationship between lower-limb lean mass and MVC plantar flexion torque ($r^2$ = 0.594; $p<0.005$). Moreover, total body fat mass was positively correlated with lower-limb lean mass ($r^2$ = 0.500; $p<0.005$) and MVC plantar flexion torque at optimum joint angle ($r^2$ = 0.332; $p<0.005$). A superior positive correlation though existed between total body mass and optimum MVC plantar flexion torque ($r^2$ = 0.458; $p<0.005$).

Conclusion: Our findings confirm that total body fat mass is associated with lower-limb fat-free mass and maximum strength in untrained women. This suggests that the antigravity muscles of the lower-limb may possibly adapt to chronically low and high levels of adiposity in a similar manner to chronic unloading or overloading of the muscles, as seen following prolonged periods of bed-rest or resistance training.

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**The Continuum of Adiposity and its Effect on Skeletal Muscle Size, Structure and Function in Untrained Young versus Old Males**

Tomlinson D.J[1]., Morse C.I.[1], Winwood K.[1], Erskine R.M[1,2]. and Onambele G.L[1].

*[1]Institute for Performance Research, Department of Exercise and Sport Science, Manchester Metropolitan University, Crewe Green Road, Crewe, CW1 5DU, UK. [2]Current address: Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, L3 3AF, UK.*

Background: In young females, obesity acts as a loading stimulus thus incrementing both skeletal muscle strength (1) and size (2). Nonetheless, the rate of change on skeletal muscle strength and size are in fact exacerbated in obese/highly adipose females (2). Studies show a gender difference in skeletal muscle volume responsiveness to BMI-induced loading (3). Therefore, we hypothesised that obesity would exacerbate the deleterious impact of ageing on skeletal muscle specific force in a male population.

Method: Thirty-four untrained healthy males categorised by age into young (Y) (mean ± SD: 25.3 ± 9.6 yrs) versus old (O) (68.7 ± 7.2 yrs) were recruited to take part in this study. Body fat was assessed using dual-emission X-ray absorptiometry. Plantar flexion maximum voluntary isometric contraction (MVC) torque was assessed in the dominant limb using an isokinetic dynamometer at 0 deg (neutral ankle position) plantar flexion. MVC joint torque was corrected for voluntary muscle activation level (assessed using the interpolated twitch technique) and antagonist muscle co-activation (assessed via surface EMG) and classified as net MVC (nMVC). Gastrocnemius medialis (GM) muscle volume (V) was measured using B-mode ultrasonography and this allowed GM intrinsic strength (nMVC/V) and specific force (SF) to be calculated.

Results: BMI was positively associated with nMVC (Y r=0.760; p=0.002; O r=0.538; p=0.021) and V (Y r=0.762; p=0.002; O r=0.471; p=0.049). BMI correlation against GM SF was positive in the younger cohort (Y r=0.594; p=0.042) but not the old (O r=0.14; p>0.05), so that the difference between the two slopes was statistically significant (Y vs. O being 0.879 N/cm$^2$/BMI vs. 0.145 N/cm$^2$/BMI; Student's *t*-statistic 2.05, p<0.05). Total body adiposity elicited similar positive associations with nMVC (Y r=0.521; p=0.056; O r=0.585; p=0.011) and V (Y r=0.708; p=0.005; O r=0.548; p=0.019). Interestingly the rate of deleterious change with increased ageing, implied through regression slopes, in terms of both intrinsic strength (-0.639 Ncm/cm$^3$/year p=0.013) and GM SF (-0.373 N/cm$^2$/year; p=0.013) was significantly steeper in the obese (i.e. high BMI). Similarly, the rate of deleterious change with increased ageing in terms of both GM V (-1.577cm$^3$/year; p<0.05) and GM SF (-0.183 N/cm$^2$/year; p<0.05) was found to be faster in the highly adipose.

Conclusion: This study demonstrates that whilst high BMI (in spite of high adiposity) has a positive loading effect on absolute torque and muscle volume in young males, in older males, presence of obesity is detrimental to skeletal muscle function. Specifically the implied rate of ageing, at the fascicular level, suggests that the endocrine effects of combined adiposity and ageing may be additive. This effect would override the mechanical loading of chronic high loads and hence impede the physiological pathways responsible for muscle function optimisation.

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**The impact of obesity on bone mineral density changes with ageing in sedentary women**

Tomlinson DJ[1], MSc, Erskine RM[1,2], PhD, Winwood K[1], PhD, Morse CI[1], PhD, and Onambélé GL[1], PhD.

[1]Institute for Performance Research, Department of Exercise and Sport Science, Manchester Metropolitan University, Crewe Green Road, Crewe, CW1 5DU, UK. [2]Current address: Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, L3 3AF, UK.

Obesity is associated with increased maximal torque [(1,2)], through an effect attributable to greater total lean mass [(Maffiuletti et al. 2007)]. Similarly, the obese have a specious bone strength advantage [(3)]. This study examined the relationship between ageing and bone mineral density (BMD), to determine whether any beneficial impact of increased load is offset by high adiposity.

94 untrained healthy women were categorised by age into young (Y) (mean ± SD: 22.4 ± 4.4 yrs) versus old (O) (64.0 ± 9.6 yrs), and by adiposity into average (<40%) vs. high (≥40%) body fat. Participants were assessed for total and segmental body composition using dual energy x-ray absorptiometry.

Total BMD decreased with ageing in the high adiposity group (-0.0029 g/cm$^2$ per year; r = 0.517, P<0.001). This subgroup in fact exhibited 12% leg (1.28 ± 0.11 vs. 1.13 ± 0.13 g/cm$^2$, p=0.001) and 8% arm (0.85 ± 0.12 vs. 0.78 ± 0.11 g/cm$^2$, p=0.001) BMD differences in Y vs O (see Figure 1). These BMD changes were in line with the lean tissue content losses (-14% in total body (p=0.01), -20% in leg (p<0.01) and -14% in arm (p=0.05) musculature of O vs Y). There were no ageing-related changes in the average adiposity group.

Our findings suggest that mechanical loading is not the primary mediator of the effects of obesity on bone strength adaptations. In fact, the increased mechanical demands of high adiposity are inadequate to modulate equivalent muscle and bone adaptations, particularly in the older person, likely leading to eventual osteosarcopenic obesity.

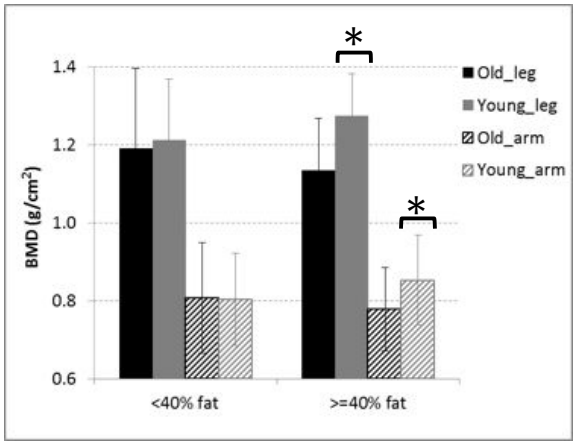


Figure 1: Age differences in appendicular BMD by adiposity. Data shown are Mean ± SD. * Denotes P<0.05.

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# PHYSICAL ACTIVITY & HEALTH QUESTIONNAIRE

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Tel (mobile): _____ Age (years): _____

Email: _____ Height: _____

Nationality: _____ Body weight: _____

What is your ethnic group? Please circle ONE section from A to F, then tick the appropriate box to indicate your background.

A) White: English ☐ Scottish ☐ Welsh ☐ N. Irish ☐ Other ☐

If other, please state here: _____

B) Mixed: White and Black Caribbean ☐ White and Black African ☐ White and Asian ☐ Other ☐

If other, please state here: _____

C) Asian: Indian ☐ Pakistani ☐ Chinese ☐ Japanese ☐ Other ☐

If other, please state here: _____

D) Black: Caribbean ☐ African ☐ Other ☐

If other, please state here: _____

E) Other ethnic background: ☐ Please state here: _____

F) I do not wish to state my ethnic origin ☐

## Blood donation

In order to investigate associations between key proteins in your blood and properties of your muscle, tendon and bone, we would like to take a small (10 mL) blood sample from a superficial vein in your arm. Before doing so, please answer the following safety questions.

1. Have you ever suffered from hepatitis or are you a hepatitis carrier? Yes ☐ No ☐
2. If you answered YES to the above question, is this Hepatitis B or Hepatitis C? _____
3. Have you ever been infected with any other blood-borne disease? __ Yes ☐ No ☐
4. Are you anaemic or receiving treatment for anaemia or iron deficiency? _____ Yes ☐ No ☐
5. Are you allergic to any of the following: Sterile alcohol pad Yes ☐ No ☐
   Plaster Yes ☐ No ☐

## Your general health

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past that may affect their ability to complete the tests and interventions required. This level of health is required for two main reasons, (1) to ensure their own continuing health and well-being, and (2) to avoid the possibility of individual health issues confounding the study outcomes.

Women: please start from Question 1; Men: please start from Question 5

1. Are you taking or have you ever taken pills as contraceptive? _____ Yes ☐ No ☐
   If yes, for how long in total have you taken "the pill"? _____

2. To your knowledge, are you pregnant? _____ Yes ☐ No ☐

3. What was your age when you had the menopause? i.e. when did your periods stop? (If you are still having your periods please put N/A). _____

4. Have you had a hysterectomy or an ovariectomy? (removal of the womb or ovaries) _____ Yes ☐ No ☐

5. Have you ever been prescribed Hormone Replacement Therapy? ____ Yes ☐ No ☐
   If YES, for how long in total have you taken "HRT" (male or female hormones)? Please specify the specific medication _____

6. Have you ever smoked? _____ Yes ☐ No ☐
   If YES, please state if you are a current or ex-smoker and how much you smoke or used to smoke _____

7. At present, do you have any health problem for which you are:
   a) on medication, prescribed (by a doctor) or otherwise _____ Yes ☐ No ☐
   b) attending (visiting) your doctor _____ Yes ☐ No ☐
   c) on a hospital waiting list _____ Yes ☐ No ☐

8. In the past two years, have you had any illness that has required you to:
   a) consult your doctor _____ Yes ☐ No ☐
   b) attend a hospital for an appointment _____ Yes ☐ No ☐
   c) be admitted to hospital _____ Yes ☐ No ☐

9. Have you ever had any of the following?
   a) Your doctor advised you not to take vigorous exercise _____ Yes ☐ No ☐
   b) Pain in your chest when you undertake physical activity? _____ Yes ☐ No ☐
   c) Central Nervous System disease, such as Parkinson, Alzheimer, Convulsions/epilepsy _____ Yes ☐ No ☐
   d) Have you any history of chest problems, such as bronchitis, asthma or wheezy chest _____ Yes ☐ No ☐
   e) Major illness, such as viral hepatitis, cancer _____ Yes ☐ No ☐
   f) Eczema Yes ☐ No ☐
   g) Diabetes _____ Yes ☐ No ☐
   h) High blood pressure _____ Yes ☐ No ☐
   i) A limb fracture _____ Yes ☐ No ☐
   j) Blood disorder, such as clotting problems, thrombosis, aneurysm, embolus) _____ Yes ☐ No ☐
   k) Head injury _____ Yes ☐ No ☐
   l) Digestive problems _____ Yes ☐ No ☐
   m) Heart problems, such as heart attack, valve disease, palpitations, angina _____ Yes ☐ No ☐
   n) Problems with bones, such as osteoporosis or osteoarthritis _____ Yes ☐ No ☐

o) Problems with joints, such as rheumatoid arthritis, any persistent pain, or any surgery on your joints _____ Yes ☐ No ☐

p) Back problems _____ Yes ☐ No ☐

q) Disturbance of balance/co-ordination, such as dizziness or balance-system dysfunction _____ Yes ☐ No ☐

r) Numbness in hands or feet _____ Yes ☐ No ☐

s) Disturbance of vision _____ Yes ☐ No ☐

t) Physical limitations, such as visual, hearing, walking problems ____ Yes ☐ No ☐

u) Thyroid problems, e.g. rapid loss or gain of weight _____ Yes ☐ No ☐

v) Kidney or liver problems _____ Yes ☐ No ☐

w) A severe allergic reaction, e.g. swelling, breathing difficulties in response to an external stimulus _____ Yes ☐ No ☐

x) Emotional or psychiatric problems _____ Yes ☐ No ☐

y) Any other illness or condition that affects your general health or interferes with your daily activities _____ Yes ☐ No ☐

z) Tested positive for or used any of the substances and/or methods on the World Anti Doping Agency banned list _____ Yes ☐ No ☐

8. If you answered YES to any of the questions above, please describe the details briefly below *(particularly in terms of whether the condition is current)* or to the investigator if you wish. This will help to confirm whether the problem was/is short term, minor, untreated or well controlled.

_____

_____

_____

9. Please provide contact details of a suitable person for us to contact in the event of any accident or emergency:
Name:
Telephone Number:

10. Are you currently involved in any other research studies at the University or elsewhere? Yes ☐ No ☐
If YES please provide details of the study:

_____

_____

## Habitual physical activity

1. What is your main occupation? _____

| | | | | | | |
|---|---|---|---|---|---|---|
| 2. | At work I sit | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |
| 3. | At work I stand | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |
| 4. | At work I walk | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |
| 5. | At work I lift heavy loads | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |
| 6. | After work I am tired | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |
| 7. | At work I sweat | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |

8. In comparison with others my own age I think my work is:
Much heavier ☐ Heavier ☐ As heavy ☐ Lighter ☐ Much lighter ☐

9. Do you play sport or exercise? Yes ☐ No ☐
If YES, which sport do you play most frequently? _____

| | | | | | |
|---|---|---|---|---|---|
| How many hours per week? | Less than 1 ☐ | 1 to 2 ☐ | 2 to 3 ☐ | 3 to 4 ☐ | More than 4 ☐ |
| Time per session (hours) | ½ ☐ | 1 ½ ☐ | 2 ½ ☐ | 3 ½ ☐ | 4 ½ ☐ |
| How many months per year? | Less than 1 ☐ | 1 to 3 ☐ | 4 to 6 ☐ | 7 to 9 ☐ | More than 9 ☐ |
| What proportion of the month? | A few hours ☐ | A few days ☐ | 2 weeks ☐ | 3 weeks ☐ | Most of the month ☐ |

If you do a second sport (or exercise class), which is it? _____

| | | | | | |
|---|---|---|---|---|---|
| How many hours per week? | Less than 1 ☐ | 1 to 2 ☐ | 2 to 3 ☐ | 3 to 4 ☐ | More than 4 ☐ |
| Time per session (hours) | ½ ☐ | 1 ½ ☐ | 2 ½ ☐ | 3 ½ ☐ | 4 ½ ☐ |
| How many months per year? | Less than 1 ☐ | 1 to 3 ☐ | 4 to 6 ☐ | 7 to 9 ☐ | More than 9 ☐ |
| What proportion of the month? | A few hours ☐ | A few days ☐ | 2 weeks ☐ | 3 weeks ☐ | Most of the month ☐ |

10. Compared with others of my own age I think my physical activity during leisure time is:
Much more ☐ More ☐ The same ☐ Less ☐ Much less ☐

| | | | | | | |
|---|---|---|---|---|---|---|
| 11. | During leisure time I sweat | Very Often ☐ | Often ☐ | Sometimes ☐ | Seldom ☐ | Never ☐ |
| 12. | During leisure time I play sport | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |
| 13. | During leisure time I watch TV | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |
| 14. | During leisure time I walk | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |
| 15. | During leisure time I cycle | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |

16. How many minutes do you walk per day to and from work, school and shopping?
Less than 5 ☐ 5 to 15 ☐ 15 to 30 ☐ 30 to 45 ☐ More than 45 ☐

I have completed the questionnaire to the best of my knowledge and any queries I may have had have been answered to my full satisfaction.

Signed: _____ Date: _____

Thank you for completing this questionnaire. All information will be kept strictly confidential.

## Baeke Physical Activity Scores

### (i)

| | Women (n=96) | | | | |
|---|---|---|---|---|---|
| | Underweight (n=15) | Normal weight (n=27) | Overweight (n=28) | Obese (n=26) | **p** |
| **Work** | 3.0 | 2.5 | 2.8 | 3.0 | p=ns |
| **Sport** | 2.5 | 2.9 | 2.6 | 2.4 | p=ns |
| **Leisure** | 2.7 | 2.6 | 2.9 | 2.6 | p=ns |

### (ii)

| | Women (n=96) | | |
|---|---|---|---|
| | Young (n=48) | Old (n=48) | **p** |
| **Work** | 2.9 | 2.8 | p=ns |
| **Sport** | 2.7 | 2.5 | p=ns |
| **Leisure** | 2.6 | 2.9 | p=0.005 |

## (iii)

| | Women (n=96) | | |
|---|---|---|---|
| | Normal Adipose | High Adipose | **p** |
| **Young (n=48)** | | | |
| **Work** | 2.9 | 2.9 | p=ns |
| **Sport** | 2.8 | 2.5 | p=ns |
| **Leisure** | 2.6 | 2.6 | p=ns |
| **Old (n=48)** | | | |
| **Work** | 2.7 | 2.9 | p=ns |
| **Sport** | 2.7 | 2.4 | p=ns |
| **Leisure** | 2.9 | 2.9 | p=ns |

## (iv)

| | Women (n=96) | | | | |
|---|---|---|---|---|---|
| | Underweight | Normal weight | Overweight | Obese | **p** |
| **Young (n=48)** | | | | | |
| **Work** | 3.1 | 2.8 | 2.8 | 3.0 | p=ns |
| **Sport** | 2.5 | 3.1 | 2.9 | 2.4 | p=0.006 |
| **Leisure** | 2.6 | 2.8 | 2.6 | 2.6 | p=ns |
| **Old (n=48)** | | | | | |
| **Work** | 2.9 | 2.7 | 2.8 | 3.0 | p=ns |
| **Sport** | 2.5 | 2.7 | 2.4 | 2.5 | p=ns |
| **Leisure** | 2.9 | 2.9 | 3.1 | 2.8 | p=ns |

## (v)

| | Men (n=34) | | |
|---|---|---|---|
| | Young (n=16) | Old (n=18) | **p** |
| **Work** | 2.8 | 2.8 | p=ns |
| **Sport** | 3.0 | 3.0 | p=ns |
| **Leisure** | 2.9 | 2.7 | p=ns |

## (vi)

| | Men (n=34) | | |
|---|---|---|---|
| | Normal Adipose | High Adipose | **p** |
| **Young (n=16)** | | | |
| **Work** | 3.0 | 2.5 | p=ns |
| **Sport** | 3.0 | 2.9 | p=ns |
| **Leisure** | 2.9 | 3.1 | p=ns |
| **Old (n=18)** | | | |
| **Work** | 2.9 | 2.9 | p=ns |
| **Sport** | 3.3 | 2.9 | p=ns |
| **Leisure** | 3.3 | 2.6 | p=ns |